The thermophysiological and ergogenic response to heat stress intervention strategies

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Thermophysiological and ergogenic response to heat stress intervention strategies.

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By

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Declaration

I declare that this thesis is my own work. It is being submitted for the degree of MSc by Research at the University of Bedfordshire.

It has not been submitted for any degree or examination in any other University or educational institute.

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Contents

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List of Tables

List of Figures

List of Abbreviations

°C	degrees centigrade
µl	microlitre
ACT	Acetaminophen ingestion
BBB	Blood-brain barrier
BM	body mass
CON	Control
CWI	cold water immersion
G-HH	glycerol hyperhydration
Hb	haemoglobin
Hct	haematocrit
HR	heart rate
HS	heat storage
HSP	heat shock protein
HSP72	inducible heat shock protein 72
ICE	Ice slurry ingestion
Kg	kilogram
LT	lactate threshold

ml	millimetre
mmol/l	millimole per litre
PC	pre-cooling
PO	power output
PV	plasma volume
RPE	rating of perceived exertion
Tb	mean body temperature
T_{core}	core temperature
T_{Rectal}	rectal temperature
TSI	thermal sensation index
T_{Skin}	skin temperature
TT	Time trial
TTE	Time to exhaustion
UOsm	urine osmolality
VO_{2max}	maximal oxygen consumption
W	watts
W-HH	water hyperhydration

Abstract

Endurance exercise in hot environments puts a great strain on both the physiological and cellular mechanisms of the body to maintain efficient heat dissipation and thermal homeostasis. Once the body is unable to dissipate more heat than is gained thermal stress increases core (T_{Core}) and skin temperature (T_{Skin}) impairing performance. Athletes and military personnel train and compete/work in many extreme environments; the utilisation of intervention strategies prior to exercise will delay the onset of fatigue and reduce thermal strain at a physiological and cellular level.

The purpose of the first experiment was to investigate the combined effect of hyperhydration and pre-cooling methods on endurance cycling performance in the heat. Five healthy males completed a 10 mile (16.1 km) self-paced time trial (TT) in a hot and humid environment (30°C & 50% RH) on 4 occasions: Glycerol hyperhydration (HH), pre-cooling (PC), glycerol hyperhydration and pre-cooling (HH+PC) and control (C). The cellular stress response was assessed via Heat Shock 70 kDa Protein 2 (HSP72) mRNA expression within leukocytes. There was a significant difference in completion time between the conditions ($p = 0.025$). On average, completion time during the PC trial was 6% faster than C ($p = 0.03$, 95% CI = -15 to - 210 s) and 4% faster than HH ($p = 0.02$, 95% CI = - 21 to -132 s). There was no significant difference in HSP72 mRNA expression between conditions ($p = 0.26$). PC via CWI alone or in combination with HH, enhanced endurance performance in hot and humid environments with no further ergogenic effect seen when HH was used in combination with PC.

In light of the findings from the first experimental chapter, experiment 2 looked at the kinetics and mechanisms of G-HH compared to hyperhydration with water (W-HH) at rest. 16 resting males' on 2 occasions: ingested one of two solutions evenly over a 90 min period. Glycerol solution (G-HH) or a water solution (W-HH). It was revealed that peak change in

plasma volume (%ΔPV) was significantly higher after G-HH (19.1 ± 6.3%) than W-HH (10.2 ± 4.5%) ($F_{1,\ 9.3}$ = 14.37, p = 0.004). G-HH effectively expanded PV more than water hyperhydration for the full 120 min observation period (p = 0.02). It is recommended that exercise and extreme environment occupational pursuits (such as military and bush firefighters), commences immediately post the 90 min ingestion period when PV expansion is highest, to delay the onset of dehydration.

The third experimental chapter investigated the potential pre-cooling action of an acute dose of acetaminophen and its comparison to established pre-cooling methods: cold water immersion and ice slurry ingestion on exercise in extreme heat. Evaluated from physiological and cellular perspective. 8 recreationally active males completed a 40 min sub-maximal run in extreme heat (40°C & 30% RH) on 4 occasions: cold water immersion (CWI), ice slurry ingestion (ICE), acetaminophen ingestion (ACT) and control (CON). There was significant reduction in T_{Rectal} (-0.48°C) and T_{skin} (4°C lower than all other conditions) after CWI compared to ICE, CON and ACT. A significant down regulation of HSP72 expression post exercise after ACT compared to CWI. ACT did not elicit a thermoregulatory reduction but did however reduce strain on a cellular level during exercise in extreme heat. CWI proved to be the most effective form of pre-cooling through the reduction of T_{Rectal} and T_{skin} prior to exercise.

These findings confirm previous research that cold water immersion alters the robust PV expansion produced by glycerol hyperhydration (Gordon, Fogarty, Greenleaf et al., 2003). Cold water immersion is the most effective pre-cooling method to reduce thermal strain and improve performance it does however lack practical application. Acetaminophen did not prove to effectively reduce thermoregulatory strain but did however reduce strain on a cellular level. These results suggest that individuals participating in prolonged exercise in hot conditions due to its practicality for use in the field further research needs to be conducted in

50 to acetaminophen’s mechanisms of action and potential to reduce thermal strain at a cellular
51 and possibly in the correct settings physiological level.

CHAPTER 1: General Introduction

Endurance exercise causes a detrimental strain on the human body; affecting the thermoregulatory, cardiovascular and neuromuscular systems (Nybo & Nielsen, 2001). The integration and interaction of these systems work to maintain whole body homeostasis (including thermal balance) which becomes increasingly challenging when hindered by exogenous factors, such as exercising in the heat (Gonzalez-Alonso et al., 2008). Exercising and physical output in the heat leads to elevated heart rate (HR), core temperature (T_{core}), skin temperature (T_{Skin}) and dehydration all of which contribute to the early onset of fatigue (Nakamura, 2011). As well as the impact on gross body physiology, thermal strain and exercise causes major stress at a cellular level (Christians et al., 2002), disturbing homeostasis and damaging cellular function (Linquist & Craig, 1988). Stimuli such as heat stress, exercise, inflammation and hypoxia stimulate the rapid expression of cytoprotective proteins – heat shock proteins (HSPs) (Yamada et al., 2008).

Individuals such as competitive athletes and military personnel regularly have to train in extreme environmental conditions, this combination of strenuous physical exertion and high ambient temperatures puts a substantial strain on the body's heat dissipation mechanisms (Gardner et al., 1996). Furthermore such individuals are known for their determination and will push their body to its physical limit which can lead to exertional heat illness (EHI). There is literature suggesting that military personnel will purposefully not drink fluid during an operation or training this is known as voluntary dehydration, it is thought that stopping to hydrate is a 'sign of weakness' (Szlyk et al., 1987). To prevent such conditions arising it is essential to utilise intervention strategies prior to the exercise to improve latent heat capacity and delay the onset of fatigue.

A plethora of research has investigated intervention strategies to improve performance in the heat, including hydration and pre-cooling (Booth et al., 1997; Goulet et al., 2006; Hasegawa et al., 2006; Kay et al., 1999; Marino, 2002; Siegel et al., 2012). Hydration strategies such as hyperhydration, act to induce a state of hyperhydration prior to endurance exercise in the heat in attempts to delay the onset of dehydration, sweating and ultimately fatigue (Nelson & Robergs, 2007). This is achieved by ingesting a large volume of fluid prior to exercise however, early research reported that water only hyperhydration was short lived due to increased diuresis (Grucza, 1987; Moroff, 1965). The addition of glycerol to hyperhydration strategies has received a lot of attention due to its water retention properties, yet results regarding its ergogenic and thermoregulatory benefit remain equivocal (Coutts et al., 2002; Goulet et al., 2006; Hitchins et al., 1999). Hydrating to a hyperhydration state prior to exercise could be highly advantageous within an occupational pursuits setting. During operation and training it is difficult to ensure all fluid replacement guidelines are enforced, hyperhydrating prior to exercise would delay the onset of dehydration and in turn symptoms of exertional heat illness (EHI).

Pre-cooling as an intervention strategy focuses on reducing T_{core} to increase the margin between basal and the 'upper safe limit'(Wegmann et al., 2012) e.g. an increase in latent heat capacity. Cold water immersion is the most effective form of pre-cooling, with a vast amount of literature reporting both significantly reduced thermal strain and ergogenic benefit (Booth et al., 1997; Kay et al., 1999; Marino, 2002). Despite its large ergogenic and thermoregulatory benefit, methods such as cold water immersion lack practical application to a field setting. Limitations to pre-cooling's application in a field setting has led to diversification of pre-cooling strategies employed, with methods such as ice slurry ingestion evidently more practical (Ihsan et al., 2010; Siegel et al., 2010). Ice slurry ingestion has been reported to significantly reduce thermal strain during exercise however the magnitude of its

pre-cooling and ergogenic effect is not considered to be as high as cold water immersion (Siegel et al., 2012).

Although more minor in nature compared to cold water immersion (Ross et al., 2013), ice slurry ingestion still has ecological limitations. A pharmacological agent with hypothermic potential would offer a method of pre-cooling with ergogenic effect and ease of application increasing ecological validity. Recent research by Mauger, Taylor, Harding, Foster, et al. (2013) reported a acetaminophen induced ~0.15°C reduction in T_{core} during exercise in the heat, which lead to an improved time to exhaustion of ~4mins. These results demonstrate acetaminophen's hypothermic potential, however further research needs to be conducted to confirm its ergogenic and thermoregulatory effect and the mechanisms by which it reduces thermal strain.

Intracellular HSPs are expressed in response to stimuli such as heat, hypoxia, exercise and inflammation (Christians et al., 2002; Kaufmann, 1990) as a universal defence mechanism for cellular protection (Linquist et al., 1988). It has been reported that an attenuation of Hsp72 expression in leukocytes runs parallel with reduced thermoregulatory and cardiovascular strain during heat acclimation (Marshall et al., 2007). This coupled with the limited research previously looking at the effect of hydration status and HSP expression (Hillman et al., 2011) indicates that HSP72 could be utilised as a molecular gauge of stress response. To utilise this as a biomarker to assess the effectiveness of intervention strategies cellular thermal stress. No previous research to date has looked at the effect of pre-cooling intervention strategies on HSP72 expression in leukocytes.

CHAPTER 2: Review of Literature

2.1 Adaption to Heat

Heat is produced in all cells of the body as mechanical or thermal energy of metabolic activity. This heat must be constantly dissipated to maintain thermal homeostasis (Cheung et al., 2000). The addition of endogenous factors such as strenuous exercise and exogenous high ambient temperature and humidity creates a detrimental physiological strain and challenge on the body's ability to regulate and maintain thermoregulation (Gonzalez-Alonso, 2012). Many organisms, including humans, appear to have a set core temperature of 37°C. It is thought this temperature is optimal where heat loss and heat production mechanisms can achieve equilibrium and thermal balance (Lim, 2008). Above this temperature proteins begin to denature, thermal inactivation of enzymes occurs and changes in the metabolic action known as the Q_{10} effect, are all observed (Miller et al., 1997). The ability of organisms to adapt to environmental stressors at a physiological and molecular level has been pivotal in evolution. Organisms including humans have evolved unique thermoregulatory systems through integration and interaction of physiological and psychological features adapted to their own environment (Cheung et al., 2000). The process of maintaining body T_{Core} and preventing heat strain involves all the systems of the body particularly, neuromuscular, cardiovascular, respiratory and central thermoregulatory systems, see figure 2.1. The most effective method to improve performance in hot environments is via acclimation; this process takes 3 - 5 days to develop heat tolerance to effectively reduce exercising Tc_{ore} and HR (Marshall et al., 2007). For individuals such as athletes and military personnel this is unrealistic and in most cases impossible due to shortage of time before competition and operations. Therefore short term strategies that can be implemented prior to exercise would be highly advantageous offering ergogenic and thermoregulatory benefit without the long term time constraints.

When the human body is unable to cope with additional heat production in extreme environments, dysfunction at cellular and physiological level arises, leading to muscle cramps, exertional heat illness (EHI) and in extreme circumstances can be life threatening (Bouchama & Knochel, 2002). It is commonly thought that heat illness occurs within the elderly with inefficient thermoregulatory systems, however, EHI is most common in the military and young athletes (Smith, 2005). EHI is a significant problem particularly within the armed forces, occurring most commonly during operation and training exercises as a result of these individuals pushing themselves to physical exertion on occasion in temperatures exceeding 50°C. The mechanisms involved in EHI are not completely understood and why it affects some and not others in the same environment exerting the same physical output is unknown. One factor that must be taken into account particularly regarding armed forces is the equipment and uniform worn during this training causing uncompensable heat stress.

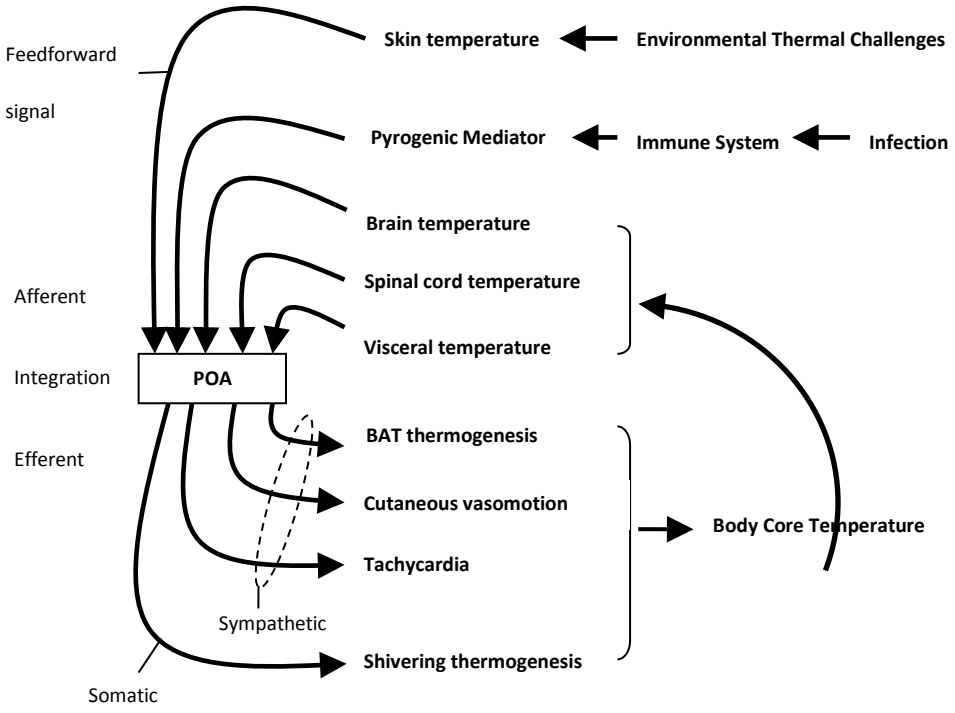


Figure 2. 1: Preoptic area (POA). A schematic of the thermoregulatory system (adapted from Nakamura, 2011).

2.2 Exercise in the heat

The fundamental action of heat stress when exercising is an early onset of fatigue, it is uncertain what the principal mechanism is involved with several hypothesises existing (Gonzalez-Alonso et al., 1999; Nielsen et al., 1993; Nybo et al., 2001). Figure 2.2 demonstrates a number of psychological and physiological factors involved in the onset of fatigue. It was first suggested that exhaustion in hot environments resulted from cardiovascular strain through the reduction in blood flow to muscles and reduced cardiac output elevating HR (Rowell, 1974). Rowell et al. (1974) investigated the cardiovascular response to exercising in hot conditions (43.3°C) compared to thermoneutral (25.6°C), reporting a significantly decreased cardiac output, stroke volume and a reduction of central blood volume of 16% in the hotter trial. All factors which will limit exercise capacity and performance in the heat. Nielsen et al. (1993) disagreed and presented evidence which showed that acclimated athletes took progressively longer to reach their core temperature limit over the 9-12 day program with no reductions in cardiac output indicating that circulatory failure was not the limiting factor to performance in the heat. Therefore, given the limiting effect T_{core} has on endurance exercise in the heat, effective intervention strategies to minimise this elevation have been employed with varying degrees of ergogenic benefit and ecological validity.

2.2.1 Critical Core Limit

The notion of a 'critical core limit' of ~39.5°C assumes that above this temperature humans are unable to continue exercising voluntarily (Gonzalez-Alonso et al., 1999; Macdougall et al., 1974; Nielsen et al., 1993). Gonzalez-Alonso et al. (1999) looked at the effect of altering

initial T_{Core} and rate of increase on time to exhaustion (TTE) performance in the heat. Despite these implemented differences, participants all fatigued at identical T_{Core} (40.1°C), leading to the conclusion that internal T_{Core} is the direct cause of fatigue when exercising in hot environments. Research conducted by Kay et al. (2001) further supported this notion in professional cyclists completing a 30 minute time trial in 30°C and 23°C. The cyclist's peak rectal temperature (T_{Rectal}) was similar between trials yet there was a 6% decrease in power output in the hotter environment. This suggests that despite being able to work harder in the temperate conditions, T_{core} will always reach a similar end point (Nielsen et al., 1993). However, studies such as Cheuvront et al. (2009) suggested T_{Core} can surpass the expected 'critical core limit' when exercising in the heat. In this case with the addition of a caffeine administration T_{Core} exceeded this expected value of 39.5°C by an average 0.2°C. Moreover, it needs to be taken into consideration that an exact end point core temperature within a laboratory setting can be dictated by ethical limitations or experimental design (Cheung et al., 2000). Kenney and Johnson (1992) used the term 'upper safe limit' in relation to T_{core} when describing exercise in the heat. This 'upper safe limit' rather than 'critical core limit' of T_{core} is supported by Selkirk and Mclellan (2001) who stated that heat stress is associated with a T_{core} approaching 40°C but this cannot be generalised for the gross population as exhaustion can occur in individuals with T_{core} of 38.5 to 39°C due to differing fitness levels (Latzka et al., 1998). Despite the very similar end point values obtained between individuals within the research mentioned previously (Kay et al., 2001; Nielsen et al., 1993); T_{Core} alone cannot be responsible for the onset of hyperthermia-induced fatigue and other mechanisms need to be taken into account. Considering this, it is important that any intervention strategy implemented before exercise does not focus on just reducing $T_{Core,}$ other factors such as hydration and sweat rate should be acknowledged to reduce thermal strain and delay the onset of fatigue through exhaustion in extreme environments.

2.2.2 Cerebral Function in Fatigue

Recent research has reported that brain temperature is highly influential in central fatigue, it has been reported it rises in correlation with elevated T_{Core} specifically, instead of peripheral parameters such as local muscle and T_{Skin} during exercise (Thomas et al., 2006). It is only in recent years that it has been possible to measure cerebral temperature during exercise and observe its relationship with strenuous exercise, heat strain and fatigue (Nybo et al., 2001). Nybo et al. (2001) hypothesised that the cause of heat-induced fatigue is located in the central nervous system and neuromuscular performance impairment is as a result of the hypothalamus sending inhibitory signals to attenuate voluntary activation of skeletal muscle. As well as signals from peripheral sensors, elevations in cerebral temperature and sensory feedback from the cardiovascular system can activate preventative action, such as reducing recruitment of muscle fibres (Cheung & Gordan, 2004). Considering the evidence surrounding the importance of brain temperature in fatigue induced through exercise in the heat, it is unlikely that fatigue is the direct result of a 'critical core limit' alone. Other factors such as motivation, exercise mode, hydration and acclimatisation should also be taken into consideration as contributors to the aetiology of fatigue (Nybo., 2008). This makes it essential that factors which can be enhanced or maintained such as hydration and T_{core} are done so through intervention strategies prior to exercise. This reduces the factors involved and potentially delaying the onset of fatigue.

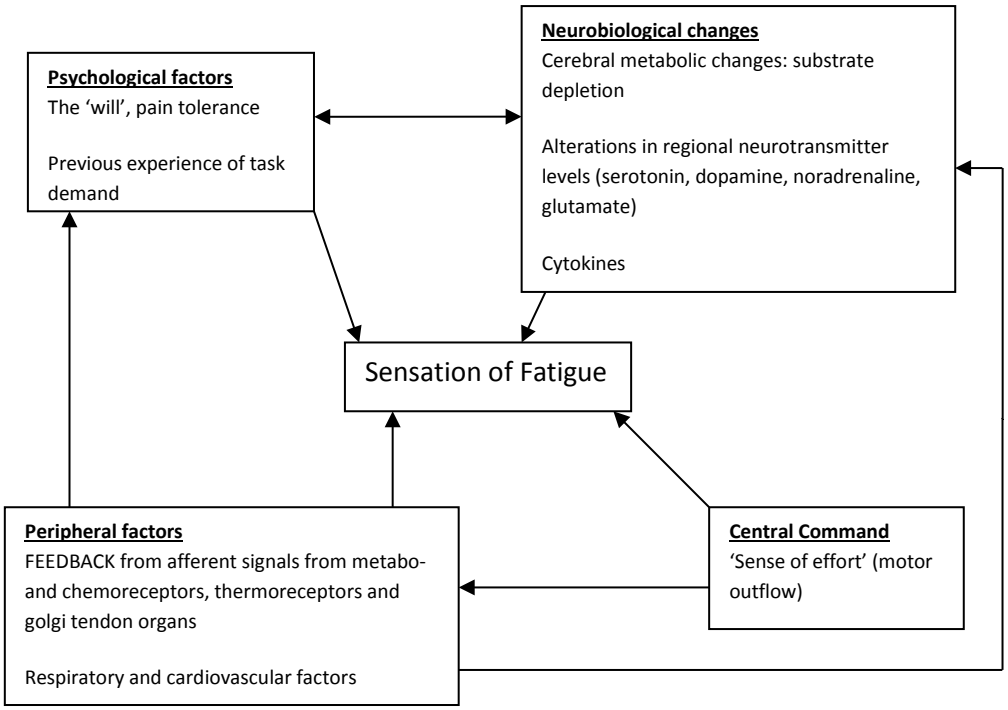


Figure 2. 2: Simplified physiological and psychological factors affecting 'the sensation of fatigue' (Adapted from Nybo (2007).

2.2.3 Effect of Heat on Performance

The combination of increased metabolic heat and the impaired heat dissipation associated with elevated environmental temperatures has a debilitating effect on performance (Hargreaves, 2008). El Helou et al. (2012) investigated the effect of environmental variables on marathon performance and reported that there was a consistent slowing of 0.03% for every 1°C increase in ambient temperature above optimum. It was concluded that air temperature is significantly correlated with performance and is the major environmental influence on performance compared to humidity, atmospheric pressure and pollution (El Helou et al., 2012). Galloway and Maughan (1997) findings further supported this, by observing that time to exhaustion was decreased by 30 min when exercising at 31°C when compared to 21°C. Exercise duration was greatest at 11°C any higher or lower than this and performance was affected in a negative manner. This effect was further exasperated with the addition of high

humidity which compounded subjects' capacity for heat loss in excess of that incurred via high ambient temperatures (Maughan et al., 2012).

When endurance exercise increases metabolic heat production, humans can maintain a thermal balance by activating heat-loss mechanisms to dissipate heat. The addition of heat and/or humidity however, causes substantial strain on the body's physiological systems ability to attenuate the detrimental effect on performance. If heat stress is allowed to continue the thermal gradient between the body and the environment will decrease, progressively impairing heat exchange (Gonzalez-Alonso, 2012). As mentioned previously Kenney et al. (1992) predicted that when heat is unable to be dissipated from the skin via sweating internal T_{Core} will reach the 'upper safe limit' within 10 minutes of exercise. If this rise in T_{Core} continues unrestricted it can limit exercise performance in recreational and competitive athletes/work output in an occupational setting subsequently resulting in the onset of EHI (Smith, 2005).

2.2.4 Mechanisms of heat transfer

There are four mechanisms of heat transfer: conduction, radiation, convection and evaporation. Conduction, radiation and convection are utilised in the early stages of increased thermogenesis while there is still a gradient within the body between the core and periphery, and between the body and its external environment (Cheung et al., 2000). Convection and conduction are the mechanisms by which heat is transferred from active muscles to the surrounding skin and through to the body's trunk and cerebral area via the circulating blood (Crandall & Gonzalez-Alonso, 2010). The process of cutaneous vasomotor control regulates the blood flow within the body. This is orchestrated by the sympathetic nervous system through vasoconstrictor and vasodilator mechanisms (Chakoudian, 2010). In hot conditions and/or the onset of exercise, vasodilation is activated to divert blood flow closer to the skin so

heat has a shorter distance to dissipate down the thermal gradient. This activation of vasodilation is mediated by cholinergic nerve cotransmission and an unknown neurotransmitter previously thought to be acetylcholine (ACh) (Edholm et al., 1957). Cholinergic nerve cotransmission are innervated blood vessels activated by the stimulation of the POA to increase skeletal muscle blood flow, in hot stress the activation instructs cutaneous resistance vessels to relax, increasing skin blood flow and cutaneous vascular conductance (Kellogg et al., 1995).

When the internal thermal gradient has decreased beyond the point where heat loss outweighs heat gain, evaporation is the main mechanism for heat transfer and dissipation. Heat is lost through the vaporisation of sweat from the skins surface cooling the blood below and can account for high rates of heat dissipation if humidity is low (Brotherhood, 2008). The extent of sweating in different areas of the body differs depending on the amount of eccrine sweat glands in the area (Kellogg et al., 1995). In dry heat evaporation can account for up to 98% of heat transfer whereas in humid environments this is progressively decreased (Armstrong & Maresh, 2003). This is due to the decrease in the thermal and water vapour pressure gradients between the environment and the body; impairing the evaporation of sweat (Gonzalez-Alonso & Calbet, 2003). It has been stated that active vasodilation and sweating have mechanistic links as they are both central heat dissipation mechanisms (Fox & Hilton, 1956), suggesting that a hydrating intervention strategy prior to exercising in the heat may benefit both hydration levels and thermoregulation.

2.2.5 Dehydration in the Heat

High rates of sweating may increase heat dissipation but it reduces blood volume (Gonzalez-Alonso, 2012). If this fluid loss (reduction in blood volume) exceeds 2% of body weight it can alter cardiovascular and thermoregulatory function, subsequently inhibiting endurance

exercise capacity (Nadel et al., 1980). Receptors in the hypothalamus are sensitive to alteration in osmolality and sodium [NA^+], reduction in both parameters indicates dehydration has occurred. The cardiovascular system will respond by reducing vascular conductance (both cutaneous and systemic) to maintain venous return (Crandall et al., 2010). Dehydration further compromises the attenuation of body T_{Core}, the associated reduction of plasma volume and stroke volume putting major strain on the peripheral blood supply to maintain central blood supply. This diversion of the blood delays the heat dissipation further increasing T_{Core} (Gonzalez-Alonso et al., 2003). There is a close relationship between T_{Core} and dehydration with reports of Tcore increasing approx 0.15°C with each 1% decrease in body weight (Sawka et al., 1985). During military operations and training it is extremely difficult to rehydrate, this is thought to be through a combination of sheer determination of the personnel and not fully understanding the importance of or implementing the hydrating guidelines set out. Therefore a hydration strategy which can elicit a higher than euhydrated state prior to exercise or training would delay the onset of dehydration and reduce the need to hydrate during the task. An intervention strategy which addresses both dehydration and decreasing latent heat capacity would be highly advantageous when exercising in the heat for prolonged periods of time either from a sports perspective or a military.

2.3 Hyperhydration

As mentioned previously exercise causes an increase in metabolic heat which in turn through heat dissipation reduces blood volume. This makes it fundamental to maintain blood volume for optimal thermoregulation and to delay the onset of dehydration. Although many hydration interventions have been published, many researchers have used glycerol as the

most common substance to maintain or increase cellular water content (Goulet et al., 2006; Latzka & Sawka, 2000; Nelson et al., 2007; O'Brien et al., 2005).

2.3.1 Biochemistry of Glycerol

Glycerol is metabolised by two major pathways once it has been phosphorylated to glycerol 3 – phosphate (see figure 2.3). 80% of glycerol is metabolised in the liver; glycerol 3-phosphate oxidised into dihydroxyacetone phosphate, which can then proceed through glycolysis to produce two adenosine triphosphates or to form glucose through gluconeogenesis (Frank et al., 1981). The second pathway is utilised by approximately 10-30% of glycerol 3-phosphate combining with two free fatty acids (FFA) to form phosphatidate, which with the addition of a third FFA forms triglyceride (Robergs & Roberts, 1997).

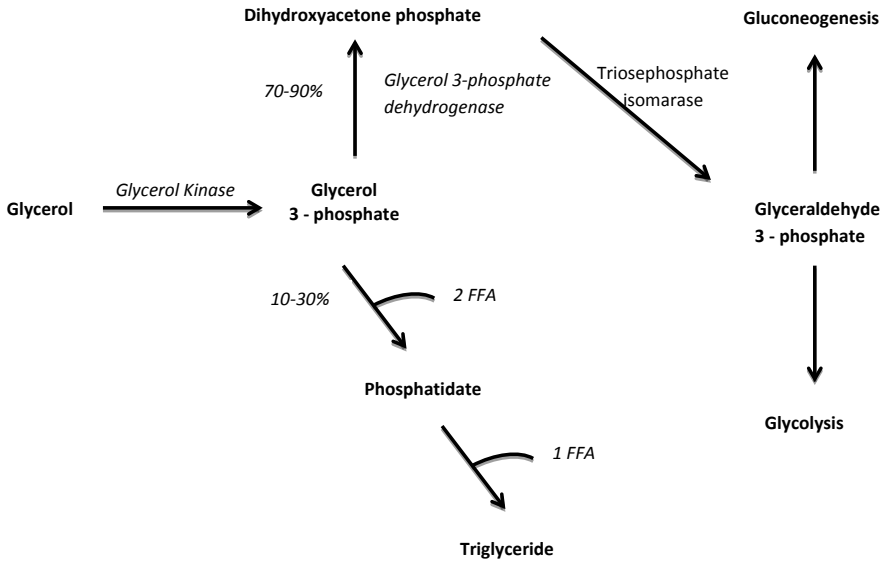


Figure 2. 3 : The metabolism of glycerol. Adapted from Hillman (2011).

The slow metabolic clearance of glycerol from the blood facilitates its hyperhydration potential. Glycerol is evenly distributed among all intracellular and extracellular fluid compartments apart from the cerebral spinal fluid and aqueous humour (Lin, 1977). The

primary mechanism of glycerol fluid retention is the establishment of an osmotic gradient through the blood – brain barrier; it is absorption increases the solute concentration of the fluid compartments compared with the outside of them (Nelson et al., 2007). Glycerol is readily absorbed and distributed among the bodily fluids, allowing the body to retain as much as 50% more than water alone. Freund et al. (1995) were among the first to demonstrate that glycerol increased fluid retention and lower rates of free water clearance; it is thought this is due to the glycerol's absorption into the tubules of the kidneys. Increasing the kidney's medullary concentration gradient, thus increasing total body water through the retention of additional water consumed (Nelson et al., 2007). Glycerol's potential to increase water retention would make it an effective pre exercise hydrating solution to maintain hydration or elevate to a hyperhydrated state in occupational and exercise performances.

Table 2. 1: Summary table of a sample of exercise after glycerol hyperhydration studies

Authors	Glycerol Dose (g · kg BM) and additional fluid (ml · kg)	Total Fluid (L)	Hydration Duration	Exercise environment	Exercise Protocol	Performance
Lyons et al. (1990)	1.0 in 3.3 of OJ and 21	1498	Consumed over 60 min, exercise commenced 90 min post ingestion	42°C (25% RH)	Treadmill walking at 60% VO2max, with 5 min rest every 30 min	↔ HR and PV, ↓ 0.9°C T_{Rectal}, performance not measured.
Montner et al. (1996)	1.2 and 26	1749	Consumed over 30 min, then additional glycerol solution at 60 min mark	24°C (26% RH)	Cycling to exhaustion at 61% Wmax	↔ T_{Rectal} and sweat rate, ↓ 4-5 bpm HR, endurance performance ↑ 21%
Hitchins et al. (1999)	1 and 22	1628	Consumed over 30 min, exercise commenced 120 min post ingestion	32°C (58% RH)	30 min fixed power output cycle and 30 min self-paced variable power output	↑ 4 bpm HR, ↔ sweat rate, T_{Rectal} and T_{Skin}. Total work ↑ 5% during variable phase.
Latzka et al. (1998)	1.2 and 29.1	1862	Consumed over 30 min, exercise commenced 30 min post this	35°C (45% RH)	Treadmill walking to exhaustion at 4-9% gradient at 1.56-1.65 m·s wearing chemical protective clothing	↔ HR, sweat rate, T_{Rectal}, T_{Skin}. Endurance time to exhaustion ↑ 13%.
Anderson et al. (2001)	1 and 20	1440	Consumed over 15 min, exercise commenced 120 min post this.	35°C (30% RH)	Cycling at 98% LT for 90 min, followed by max effort for 15 min	↓ 7 bpm HR, ↓ 0.4°C T_{Rectal}, ↔ T_{Skin} (tended to be ↓ 0.4°C throughout). Work performance ↑ 5%.

Coutts et al. (2002)	1.2 and 25	1955	Consumed over 60 min, exercise commenced 70 min post this	Hot: 30.5°C (46% RH) Warm: 25.4°C (52% RH)	Olympic distance triathlon (1.5 km swim, 40 km cycle, 10 km run)	↑ 5% PV, ↔ sweat rate. ↓ 11% run time, ↓ overall for triathlon time.
Marino et al. (2003)	1.2 and 21	1655	Consumed over 150 min, Exercise commenced immediately after.	34.5°C (63.4% RH)	Cycling for 60 min aiming to complete the furthest distance possible, 1 min sprints at 10,20, 30 , 40, 50 and 60 min	↑ 10-20 bpm HR during sprints, ↑ 50% sweat rate, ↔ T_{Rectal} and T_{Skin}. ↓Total distance cycled.
Wingo et al. (2004)	1 and 2.8% of BW	2153	Consumed over 120 min, exercise commenced 35 min after this	30°C	3 x 10 mile loop mountain bike race (8 min break between loops)	↔ HR, sweat rate, T_{Rectal} and T_{Skin}. Time to complete final loop ↓ 9% but not significant.
Goulet et al. (2006)	1.2 and 26	1781	Consumed over 110 min, exercise commenced 10 min after this	25°C (38-42% RH)	Cycling at 65% VO_{2max} for 120 min, 5 min break and then an incremental cycle to exhaustion	↔ HR, sweat rate and T_{Rectal}. ↔ in performance.
Dini et al. (2007)	1 and 28.5	2500	Consumed over 90 min, exercise commenced 180 min after this	36°C (30% RH)	Rowing incremental protocol: 6 x 3 min blocks, with 1 min rest between each, starting at 250 W and increasing to 400 W. Repeated 3 times.	↔ HR. Work at anaerobic threshold during 2nd and 3rd performance blocks was maintained significantly more.
Goulet et al. (2008)	1.2 and 26	1776	Consumed over	26°C (55% RH)	Cycling at 65% VO_{2max} for 120 min with 5 x 2min intervals at 80% VO_{2max} performed every 20 min. Then incremental TTE	↓HR, ↔ sweat rate and T_{Rectal}. ↑ 12.5% endurance performance and PPO 5%.

↑ (Increase), ↓ (Decrease), ↔ (No change), HR (Heart rate), m · s (metres per second), PPO (Peak power output), PV (plasma volume), T_{rectal} (rectal temperature), T_{skin} (Skin temperature), TTE (Time to exhaustion), VO_{2max} (Maximal oxygen uptake).

2.3.3 Glycerol Hyperhydration and Performance

Research into glycerol hyperhydration and its effect on performance and physiological functions can been seen in table 2.2. The majority of the studies have reported positive enhancements of performance. The main consensus of research reports an improvement in performance after G-HH ranging from 5-21%. For instance, Montner et al. (1996) found that glycerol hyperhydration increased cycling TTE by 21% compared to water alone. Similarly, Coutts et al. (2002) observed decreased completion time of a triathlon in hot conditions (30°C). Despite this, some studies have observed no change in performance (Goulet et al., 2006; Marino et al., 2003), this may be due to the long ingestion period utilised by both studies (110 - 120 min) causing the fluid retention and plasma volume (PV) expansion to peak and begin to decrease before the exercise protocol has even begun.

As well as the effect on hydration status and fluid retention it was thought that G-HH will reduce cardiovascular and thermoregulatory strain. A number of studies support this such as Anderson et al. (2001), heart rate was on average 7 bpm lower after glycerol hyperhydration compared to water alone, moreover T_{Rectal} was reported to be 0.4°C lower by the end of the exercise bout. Lyons et al. (1990) also details reduction in T_{Rectal} and increases in sweat rate during 90 min of walking in 42°C. However the majority of studies found no cardiovascular or thermoregulatory effect of G-HH (Goulet et al., 2006; Hitchins et al., 1999; Marino et al., 2003; Montner et al., 1996). Methodological differences may account for the conflicting results obtained, for example some studies were conducted in hot environments (Hitchins et al., 1999; Latzka et al., 1998; Lyons et al., 1990) while others were in temperate (Montner et al., 1996), this would affect whether a thermoregulatory response could be seen from the glycerol hyperhydration. Furthermore several studies allowed hydration during the exercise protocol while others did not, distorting whether an improvement was directly as a result of the primary G-HH ingested. There is also difference in study's control; some used water

hyperhydration as a placebo comparison (Lyons et al., 1990; Riedesel et al., 1987) while other studies allowed no fluid ingestion at all prior to exercise (Freund et al., 1995; Latzka et al., 1998).

2.3.4 Glycerol Hyperhydration Dosage

Many studies have observed the fluid retention potential of glycerol hyperhydration at rest and during exercise (Freund et al., 1995; Lyons et al., 1990; Montner et al., 1996). Riedesel et al. (1987) was the first to observe glycerol as an agent to enhance fluid retention, looking at the dose-response relationship for glycerol doses of 0.5, 1 and 1.5 g·kg BM. All the doses increased water retention, however 0.5 g·kg BM did not increase water retention higher than water alone, additionally 1.5 g·kg BM did not further increase water retention significantly compared to 1 g·kg BM. This indicates that glycerol dose needs to be between 1 g·kg BM and 1.5 g·kg BM for maximal effect. Further research supports this dosage from Van Rosendal et al. (2010) and from the summary table 2.1 presented above a dosage of 1.2 g·kg BM of glycerol proved to result in the optimal hyperhydration and should be administered with the addition of 26 ml·kg BM of water. As well as glycerol dosage, there is large heterogeneity in research regarding the period of time between ingestion and exercise commencement. Glycerol ingestion has been reported to induce hyperhydration for up to 4 hours, it is very difficult to determine the peak hyperhydration level due to the different methods of administration and timing of measurement utilised between studies. Hitchins et al. (1999) and O'Brien et al. (2005) have reported that the minimal amount of time required to observe the enhancement of glycerol hyperhydration is 90 min. Exercise should start as early as possible after this ingestion period to maximise fluid retention. Despite the supported effective potential of G-HH to improve fluid retention and performance, restrictions in place need to be taken into consideration.

2.3.5 WADA

A significant event occurred in recent time regarding the use of glycerol; due to glycerol's ability as a plasma expander WADA added glycerol to the prohibited substances list under 'diuretics and other masking agents' in section S5 in 2010, banning it both during and out of competition (Wada, 2012). All plasma expanders are prohibited due to their ability to promote fluid retention, increasing volume in the vascular space causing haemodilution. This can then potentially dilute any concentrations of banned substances to levels that are insufficient for detection. Thus it is important that athletes do not ingest dosages of glycerol high enough to induce hyperhydration, suggesting that future research should focus on glycerol use for recreational athletes and occupational pursuits/military.

Despite its fluid retention potential, glycerol hyperhydration's thermoregulatory effect still remains equivocal. Regardless of this, glycerol hyperhydration could still provide a valuable alternative for athletes and occupational pursuit individuals who find it extremely difficult to hydrate during training or operation. An intervention strategy with a more efficient thermoregulatory and ergogenic effect such as pre-cooling may be more advantageous to improve tolerance to the heat and exercise performance. With the potential to combine the two interventions to utilise both strategies' differing effects to improve performance in heated conditions could result in optimum performance in extreme environments.

2.4 Pre-cooling

Pre-cooling strategies have been the focus of a plethora of investigations looking to reduce body temperature and increase heat storage capacity over the last three decades (Booth et al., 1997; Cotter et al., 2001; Quod et al., 2008). The first study to observe the effect of pre-cooling on performance was Bergh and Ekblom (1979), who observed the effect of swimming in cold water at 13˚C for 20 min prior to a time to exhaustion cycling trial in ambient temperature. No significant improvements in performance were reported; potentially due to the exercise being conducted in ambient temperature and the pre-cooling involving exercise which increases metabolic heat production prior to starting. Despite these findings this study lead to extensive research to be conducted in this field. The fundamental mechanism of pre-cooling is achieved through the reduction in body temperature to increase the margin between initial core temperature and high elevation in core temperature which has a detrimental effect on performance, additionally to decrease heat stress and delay the onset of sweat threshold (Olschewski & Bruck, 1988).

2.4.2 Pre-cooling and Exercise Type

The effect of pre-cooling depends somewhat on the form of exercise undertaken. Although the majority of pre-cooling research focuses on endurance exercise performance particularly time trials (Duffield et al., 2010; Kay et al., 1999; Quod et al., 2008) a number of studies have looked into its effectiveness for intermittent sprint performance (Castle et al., 2006; Duffield et al., 2003) resulting in little of no improvement on performance compared to those found for endurance performance. Even within endurance exercise there are different protocols utilised which can have different results, the main types being: fixed tests (Quod et al., 2008), time to exhaustion (Bogerd et al., 2010; Lee. & Haymes., 1995) and time trials (Ihsan et al.,

2010; Kay et al., 1999). The highest performance enhancement has been found in time to exhaustion trials, this is thought to be because of the large variability in performance ability compared with time trial protocols (Jeukendrup et al., 1996). On the other hand time trials are more applicable to the demands of a competitive sport in its field setting where as a time to exhaustion is limited to showing exercise capacity.

2.4.3 Methods of pre-cooling

External pre-cooling techniques create a micro-environment, increasing the thermal gradient between core and periphery and decreasing the thermoregulatory strain associated with exercising (Booth et al., 1997). Different external pre-cooling methods are designed to principally reduced skin temperature such as ice vests, evaporative cooling shirts and ice packs (Castle et al., 2006; Cheung & Robinson, 2004), whereas others aim to reduce both T_{skin} and T_{Core} such as cold water immersion and cold showers (Hessemer et al., 1984; Vaile et al., 2008). More recent research has investigated the efficiency of internal pre-cooling via ice slurry ingestion, cold water ingestion cold air inhalation (Geladas & Banister, 1988; Lee et al., 2008; Mauger, Taylor, Harding, Wright, et al., 2013; Siegel et al., 2010). Internal pre-cooling has been defined as the process of taking in a cold medium into the body through the mouth or nose (Ross et al., 2013), the benefit of such methods is their logistical simplicity and practicality in a sport field setting. As well as cooling the athlete, ice slurry and cold water ingestion have the additional advantage of maintaining fluid balance and hydration status. Another form of internal pre-cooling which has recently been investigated is acetaminophen, with limited research into this pharmacological agent's pre-cooling benefit (Mauger, Taylor, Harding, Wright, et al., 2013) it has been implied that it has hypothermic action which can potentially reduce thermoregulatory strain improving performance.

2.4.4 Cold water immersion - mechanisms

The mechanism of action of cold water immersion centres on the body's effort to maintain T_{Core}, primarily this is achieved through reducing skin blood flow to facilitate vasoconstriction (Burton & Bazett, 1936). If the water is a temperature below 33°C, vasoconstriction will not be able to maintain core temperature for a long period of time; this will lead to an expansion in metabolic heat production explaining the subsequent increase in core temperature. However, after a short period heat loss will exceed heat production and core temperature will dramatically decline. Young et al. (1987) suggests during cold water immersion the combined effect of hydrostatic pressure and cold stress would induce intense vasoconstriction, thereby reducing the vascular volume of the peripheral vessels. The circulation of cold blood and increased heat storage capacity resulted in blunting of body temperature elevation (Gonzalez-Alonso, 2012).

2.4.5 Literature

Visual inspection of Table 2.2 indicates that cold water immersion is the most effective method of reducing thermoregulatory strain which in turn significantly improves endurance performance in a heated environment (Booth et al., 1997; Kay et al., 1999; Marino, 2002). There is however large heterogeneity in methodological design and exercise protocols utilised in cold water immersion making it difficult to establish the duration and temperature for optimal performance benefits. It has previously been stated that to have significant effect on T_{Core} during exercise, cold water immersion should be maintained for 30-60 mins (Quod et al., 2008). Booth et al. (1997) supports the use of 60 mins of cold water immersion in 24°C which resulted in a significant reduction in rectal temperature by 0.7°C and 30 min TT performance was increased by on average 304 m. Duffield et al. (2010) however utilised only a 20 min immersion period in 14 °C reporting a small but significant reduction in T_{Core} of at least 0.2°C throughout the 40 min TT and an increase in power output by 20 W respectively.

These results imply that a long duration of cold water immersion elicits a greater pre-cooling effect but a shorter duration at cooler temperatures still reduces T_{Core} and is more advantageous in sport settings. Cold water immersion has been proven to be an effective pre-cooling method in laboratory settings, but lacks practicality for field settings and sporting competitions.

Other external methods such as cold air exposure (Hessemer et al., 1984; Schmidt et al., 1981) which was the focus of early research into pre-cooling take a long period of time (90 min) to gain significant reduction in T_{Core} (1°C) and can result in involuntary shivering utilising metabolic heat production (Schmidt et al., 1981). More practical methods have been explored, the most published being the ice vest (Castle et al., 2006; Quod et al., 2008). Results proved this method to be less effective at reducing T_{Core} (0.2-0.7°C) with the added concern over the vests weight and its use during warm ups adversely affecting necessary temperature related mechanisms such as increased nerve-conduction rate, decreased stiffness and increased anaerobic energy provision (Bishop, 2003). The development of a more ecologically valid but effective pre-cooling mechanism was essential.

Table 2. 2: Summary table of a sample of exercise following pre-cooling studies

Authors	Exercising Environment	Pre-cooling method	Exercise Protocol	Physiological response	Performance
EXTERNAL Schmidt and Bruck (1981)	18°C	Cold room, temperature range 0–18°C (90 min)	Stage test until exhaustion on a cycle ergometer	↓ 1 °C T_{rectal}; ↓HR; ↔peak oxygen uptake	TTE was similar between conditions
Hessemer et al. (1984)	18°C	Cold room, temperature range 0–18°C (90 min)	60 min time trial on a cycle ergometer	↓4.5 °C T_{skin}; ↓1 °C T_{Body}; ↔HR	Cold air increased mean work rate by 6.8% vs control
Booth et al. (1997)	32°C (60% RH)	Cold water application [whole body] 24°C (60 min)	30 min time trial on a treadmill	↓0.7 °C T_{rectal}; ↓5.9 °C T_{Skin}; ↓HR	Cold water increased distance run by 304 m vs control
Gonzalez-Alonso et al. (1999)	40°C (19 % RH)	Cold water application at 17°C (30 min)	Open-end test on a cycle ergometer at 60% VO2max	↓1.5 °C Tcore; ↔ BML; ↔HR	Cold water increased TTE by 27% vs control
Kay et al. (1999)	31°C (60% RH)	Cold water application [whole body], until skin temperature was reduced by 5–6°C (60 min)	30 min time trial on a cycle trainer	↓5 °C T_{skin}; ↓1.6 °C Tbody; ↔HR	Cold water increased performance by 5.7% vs control
Drust et al. (2000)	21°C (72% RH)	Whole-body showering [28–24°C] (60 min)	Soccer-specific exercise on a treadmill for 90 min	↓0.6°C T_{rectal}; ↔Oxygen consumption; ↔HR	Energy expenditure was similar between conditions
Cotter et al. (2001)	35°C (60% RH)	Ice vests, cold air [3°C] and with (LW) or without leg cooling (LC) (45 min)	2 stages on cycle ergometer (30 min), 20 min 65% *V*O2peak then a 15-min time- trial	LC: ↓T_{rectal}; ↓T_{skin}; ↓HR LW: ↓Trectal; ↓HR	With leg cooling increased PO by 17.5% without increased by 16% vs control
Mitchell et al. (2003)	38°C (40% RH)	Ambient temperature 22°C, fan cooling with water spraying (20 min)	Open-end test on a treadmill At VO2max	↓1.1 °C T_{rectal}; ↓1.9 °C T_{skin}; ↓ 15% HR	TTE increased by 8%

Cheung et al. (2004)	22°C (40% RH)	Cooling vest 75 min or until rectal temperature was reduced by 0.5°C	30 min intermittent sprint on a cycle ergometer	↓0.5 °C T_{rectal}; ↓1.8 °C T_{Skin}; ↔HR	PPO was similar between conditions
Castle et al. (2006)	33°C (51% RH)	Ice vest, cold water application [17.8°C] or ice packs (20 min)	cycling intermittent sprint protocol (40 min)	Vest: ↓0.3 °C T_{rectal}; Cold water: ↓0.3 °C T_{rectal}; ↓0.7°C T_{mus} Ice packs: ↓0.2 °C T_{rectal}; ↓1°C T_{mus}	Ice packs increased PPO by 4%
Quod et al. (2008)	34°C (41% RH)	Cold water application [whole body at 29–24°C] (30 min) + cooling vest (40 min)	Fixed intensity on a cycle ergometer: 20 min at 75% VO2max, then self-paced time trial	↓0.7 °C T_{rectal}; ↓8.1 °C T_{skin}; ↔HR	Combination increased time trial by 3.8-2.3% vs control.
Duffield et al. (2010)	33C (50% RH)	Cold water application [lower body at 14° C] (20 min)	40 min time trial on cycle ergometer	↓0.2°C T_{Rectal}; ↓ 1°C T_{Skin}; ↔ HR	Cold water application increased PPO by 20 W
INTERNAL Lee et al. (2008)	35°C (60% RH)	3 · 300mL cold drinks [4°C] (30 min)	Open-end test on a cycle ergometer at 65% VO2max	↓0.5 °C T_{rectal}; ↔T_{skin}; ↓HR	Cold water ingestion increased TTE 23 – 26%
Ihsan et al. (2010)	30°C (74 % RH)	Ingestion of crushed ice [6.8 g · kg] (30 min)	40 km time trial on a cycle ergometer	↓1.1 °C T_{gi}; ↔ T_{skin}; ↔HR	Ice slurry ingestion improved performance by 6.5%
Siegel et al. (2010)	34°C (55% RH)	Ingestion of crushed ice [7.5 g · kg] (-1°C) (30 min)	progressive treadmill run to volitional exhaustion	↓0.66 °C T_{rectal}; ↓0.3 °C T_{skin}; ↔HR	Ice slurry ingestion increased TTE 19%
Siegel et al. (2012)	34°C (52% RH)	Ingestion of crushed ice [7.5 g · kg] (-1°C) (30 min)	progressive treadmill run to volitional exhaustion	↓0.4°C T_{rectal}; ↔ T_{skin}; ↔HR	ICE similar to CWI. ICE increased TTE by 13% vs control
Mauger, Taylor, Harding, Wright, et al. (2013)	30°C (50% RH)	acetaminophen ingestion [20 mg · kg] (45 min)	Time to exhaustion on cycle ergometer	↓0.15 °C T_{rectal}; ↓0.47 °C T_{skin}; ↔HR	acetaminophen increased TTE by 18% compared to control

↑ (Increase), ↓ (Decrease), ↔ (No change), BML (Body mass loss), HR (Heart rate), ICE (ice slurry ingestion), PPO (Peak power output), RH (Relative humidity, T_{Rectal} (rectal temperature), TTE (Time to exhaustion), T_{Skin} (skin temperature), VO_{2peak} (Maximal oxygen uptake), VO_{2max} (Maximal oxygen uptake).

2.4.6 Ice Slurry Ingestion - mechanisms

Ice slurry ingestion's mechanism of action is by reducing the temperature of the stomach in turn reducing the thermal load (Siegel et al., 2012). The change of ice to liquid requires 333.55 J of thermal energy this large heat absorption is known as 'enthalpy of fusion' of ice, creating a greater cooling effect than water (Merrick et al., 2003). The combination of solid and liquid H_2O thermodynamic properties has the potential to reduce heat retention and a greater heat storage capacity.

It was previously stated that 1 – 1.7 L of cold water needed to be ingested to result in core temperature reductions of 0.6 to 0.8°C (Imms & Lighten, 1989) however Ihsan et al. (2010) achieved a similar cooling effect (0.5°C) after a considerably smaller volume of crushed ice. The use of ice slurry ingestion as a pre-cooling method has received great research attention in recent years with results conveying significant reductions in core temperature (Ihsan et al., 2010; Siegel et al., 2010; Siegel et al., 2012). However, it is important to take into consideration the equipment used to measure core temperature. Many studies used ingestible core temperature measurement pills, the influence of the ice slurry directly on the telemetric pill can influence results giving unreliable measurements (Wilkinson et al., 2008). Ice slurry ingestion was proven comparable in improving time to exhaustion performance to cold water ingestion (Siegel et al., 2010). Ihsan et al. (2010) also reported a reduction in gastrointestinal temperature of 1.1°C and a 6.5% improvement in 40 km cycling performance after ingestion of 6.8 $g \cdot kg^{-1}$ compared to tap water ingestion. Several studies have reported a significant elevation in T_{core} at the point of exhaustion after ice slurry ingestion compared to control and cold water immersion (Siegel et al, 2012., Siegel et al 2010). Siegel et al. (2012) found a 0.28°C increase, implying this rise to be a consequence of ice slurry altering brain temperature and the possibility that the critical temperature originates in the brain rather than the core (Caputa et al., 1986).

2.4.7 Acetaminophen - mechanisms

Acetaminophen (paracetamol) is one of the most widely used and available non-prescription medications worldwide. As well as being a common analgesic, acetaminophen also has antipyretic capabilities (Ayoub et al., 2004). Primary research into acetaminophen centred on clinical trials looking at acetaminophen's antipyretic effect on acute ischemic stroke patients (Den Hertog et al., 2009; Dippel et al., 2001; Kasner et al., 2002; Koennecke & Leistner, 2001). The severity of ischaemic neuronal injury after a stroke is critically influenced by this elevation in body temperature in the first 4 – 6 hours post-acute stroke (Boysen & Christensen, 2001); with enforced mild reductions in brain temperature being shown to reduced post-ischemic neuronal necrosis and connected to higher mortality rates (Castillo et al., 1994). Kasner et al. (2002) and Dippel et al. (2001) both reported reductions in body temperature (0.22 – 0.4°C) in afebrile stroke patients after acetaminophen ingestion. It should be noted however, that these trials involved dosages of 650 -1000 mg 4-5 times daily which is much higher than any therapeutic dose ethically allowed within sport and exercise. Nevertheless, these results support the use of acetaminophen as a potentially beneficial hypothermic agent in the short term.

The mechanisms of acetaminophen and pathways for hypothermia are uncertain, it is widely accepted that it occurs through the inhibition of prostaglandin synthesis (see figure 2.4). Prostaglandin E_2 (PGE_2) are localised factors which are associated with pain, fever and inflammation, fundamentally they are the final mediator of a fever within the brain (Boulant, 2000). The commonly recognised pathway of inhibiting PGE_2 by acetaminophen is through inhibition of COX-2 and COX-1 through the metabolism of the peroxidise function of these isoenzymes (Graham et al., 2013).

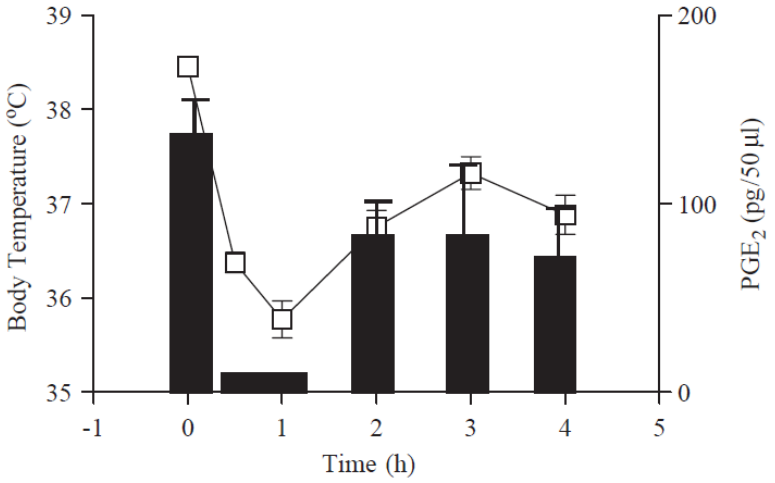


Figure 2. 4: shows the parallel reduction in PGE_2 concentration with the hypothermic effect in the brain. The line graph represents average body temperature and the histogram represents brain PGE_2 levels (Botting & Ayoub, 2005).

2.4.8 Pyrogenic Effect of Exercise

Although the research into acetaminophen's antipyretic effect has proven it has the potential to reduce fever induced elevated body temperature (Kasner et al, 2002, Dippel et al, 2001), it was unknown whether it would be effective at attenuating elevated core temperature during exercise in the heat in healthy fit individuals. It was previously though that acetaminophen's hypothermic effect could not be replicated in healthy individuals as a proliferation in PGE_2 was needed to induce antipyresis. However it has been reported that exercise has a pyrogenic effect in humans; during the course of exercise body temperature is elevated and thresholds for heat loss activity increases developing a fever like response (Bradford et al., 2007). This increase in body temperature stimulates the release of cytokines such as IL-6 and IL-1b parallel to that seen in febrile stroke patients (Martin et al., 1997; Northoff et al., 1998). This literature supports the theory that an antipyretic such as acetaminophen has the potential to limit the pyrogenic effect of exercise and attenuate the elevation in body temperature and in turn benefit performance.

There is very little research into the thermoregulatory effect of acetaminophen in healthy individuals during exercise performance. Mauger, Taylor, Harding, Foster, et al. (2013) conducted a study looking at the thermoregulatory effect of acetaminophen on thermoregulatory response and time to exhaustion (TTE) performance in ambient temperature. The results were small but significant with an average reduction in core temperature of 0.15°C in comparison to placebo during the first 60% of the TTE and significantly improved TTE of on average 200 sec after acetaminophen. A limitation of this study is that T_{core} was not measured from baseline and throughout acetaminophen ingestion period, this would have shown if the antipyretic drug acts as a pre-cooling agent reducing body temperature prior to exercise or attenuates the rise during the exercise protocol.

2.4.9 Future Direction of Pre-cooling

Although many of the commonly researched pre-cooling strategies such as cold water immersion has significant effect on thermoregulation and endurance performance, this can be outweighed by the lack of ecological validity, cost, time consumption and transportation implications. Internal methods such as ice slurry ingestion has made a step towards a more practical form of pre-cooling with easier application in field setting, improving ecological validity but does not prove as effective at reducing thermoregulatory strain. A pharmacological agent with the ability to significantly reduce or attenuate core temperature offers a method of pre-cooling in a controlled and reproducible manner with ease of application. Current research although small, has shown positive results for acetaminophen on thermoregulatory system when exercising in the heat. If more research shows similar results and can substantiate acetaminophen as an effective pre-cooling agent, it could be a method that combines a significant reduction in body temperature and improvement in performance with high ecological validity. A pharmacological agent with the ability to

significantly reduce or attenuate T_{core} would offer a method of pre-cooling in a controlled and reproducible manner with ease of application.

2.5 Molecular Mechanisms

Recent research has gained insights into the molecular base underlying thermoregulatory response. Temperature change is a major stress to the majority of organisms; sensory thermoreceptors and molecular processes are utilised to detect temperature alterations and adapt biochemical processes accordingly in living cells (Digel, 2011). These can transduce a physiological change such as temperature into a biologically significant signal such as inhibition/stimulation of genes (Bandell et al., 2007). When cells are exposed to elevated temperature it can have a detrimental effect on transcription and translation. Temperature-controlling processes need to be expressed to alleviate this stress the most common and researched being heat-shock proteins (Yamada et al., 2008).

2.5.1 Heat Shock Response

Heat shock response was first discovered by Ritossa (1962), they observed an increase in temperature by a few degrees in *Drosphila* salivary gland, inducing the synthesis of a small number of proteins. Heat shock response is a universal homeostatic mechanism to protect cellular and entire organism function from the effects of environmental stress factors (Linquist et al., 1988; Morimoto et al., 1994). This is achieved via the rapid induction of gene encoding of cytoprotective proteins – heat shock proteins (HSPs). There are always a number of heat shock genes within the cells in non-stressful conditions for development and differentiation (Santoro, 2000). HSPs are significantly expressed in response to stimuli such as: hyperthermia, exercise, hypoxia, radiation, inflammation and ischemia (Christians et al., 2002; Kaufmann, 1990). The most thermosensitive of which are HSP72 and HSP90 which

participate in stabilising protein structure, binding and unfolding proteins and enabling the refolding when intracellular conditions stabilise (Magalhaes et al., 2010). HSP72 has been reported to be mainly stress-induced with very low concentrations being present in unstressed cells (Milarski & Morimoto, 1989). Intracellular and extracellular HSP72 have varying functions. Intracellular HSPs activate an endogenous anti-inflammatory system within the cell which reduces injury and necrosis of cells not directly involved whereas extracellular HSPs are involved in a pro-inflammatory immune response in serum/plasma (Yamada et al., 2008). It is still unclear what factors mediate the activation of HSP's during exercise, it is generally accepted that related factors such as the decreased glycogen storage (Fehrenbach et al., 2001), increased stress hormone levels (Locke et al., 1990) and muscle hyperthermia (Oishi et al., 2002) stimulate HSP expression. Furthermore, reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as hydrogen peroxide and superoxide are released by leukocytes in response to endurance exercise (Niess et al., 1999) these result in oxidative stress and stimulate HSP expression.

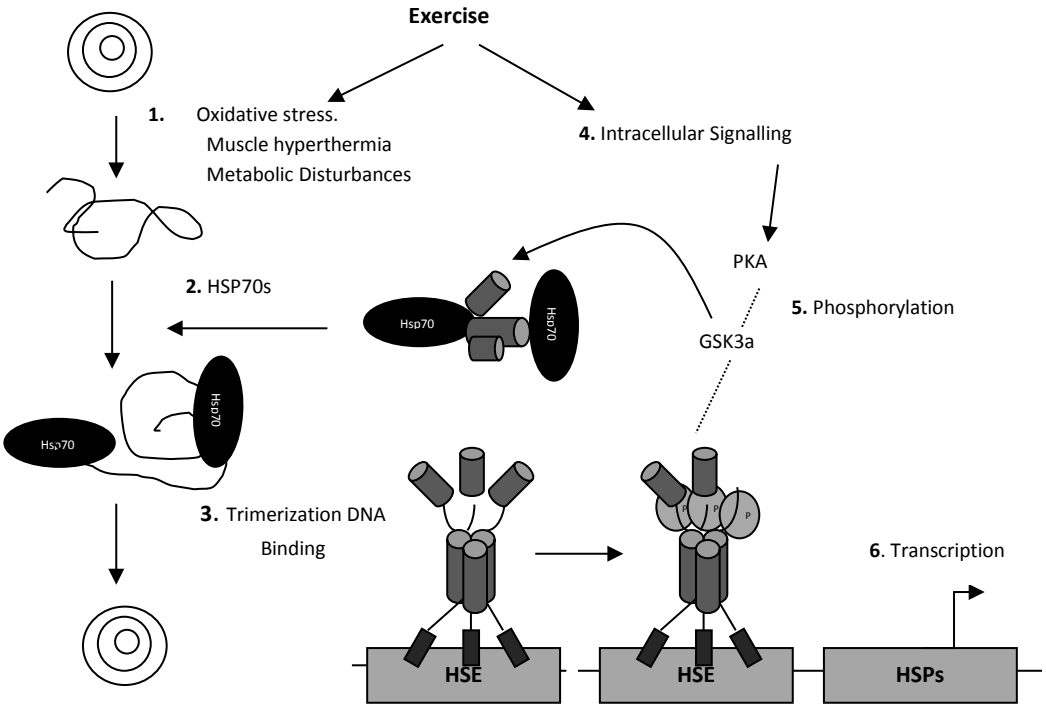


Figure 2. 5: Activation and control of the heat shock response after exercise. HSE (heat shock element), HSP's (heat shock proteins), PKA (protein kinase a), GSK3a (HSF1 repressive factor). Adapted from Noble et al. (2008).

2.5.2 Heat Shock Protein Response to Stressors

HSP70 are bound to heat shock factor (HSF1) within inactive cells (refer to figure 2.5). After exposure to stressors and the commencement of protein denaturation HSP70s dissociate from HSF1 while they aid the refolding of unfolded and misfolded proteins (Baler et al., 1992). HSF-1 then goes through several events such as localization, trimerization and DNA binding to the heat shock element (HSE), this process initiates the transcription of the HSP70 gene through phosphorylation and dephosphorylation to further express HSP70. Once the exercise-heat related protein damage is alleviated, HSP70 rebinds with HSF1 repressing its transcriptional activity and returning to an inactive monomeric state (Noble et al., 2008).

2.5.3 Heat shock Proteins and Exercise

Exercise in general has been reported to increase the expression of HSPs within the muscle and leukocytes however, several factors can affect the rate of change including type of exercise (Morton et al., 2006), intensity of exercise (Liu et al., 2000), environmental temperature (Fehrenbach et al., 2001) and training status (Fehrenbach et al., 2000). Early research into HSPs particularly HSP70 consistently reported elevations in expression after heat stress of varying durations and intensities (Blake et al., 1990; Locke et al., 1990). Skidmore et al. (1995) reported in rats that HSP72 is expressed independent of T_{core} indicating that other factors contribute to its expression. As illustrated in table 2.3 varying HSP72 expression levels have been reported in studies researching the effect of exercise on HSP70. This may be down to differing exercise durations and intensities employed. Expression of exercised induced HSP72 in leukocytes is reported to be highly influenced by exercise intensity (Shin et al., 2004). Fehrenbach et al. (2000) investigated the effect of a half marathon and reported significant increments of HSP72 in leukocytes up to 24 h post exercise, whereas Chang et al. (2010) used a higher intensity (60% VO_{2max}) protocol for only 20 min resulting in no change in HSP70 expression levels. It has also been reported that individual's fitness level affects both basal and exercise induced HSP70 expression (Febbraio & Koukoulas, 2000; Shin et al., 2004). Shastry et al. (2002) reported significantly lower baseline HSP levels in trained individuals compared to untrained, results supported by Shin et al. (2004) findings. This down regulation of HSP-positive leukocytes may be a tolerance mechanism to exercise as a result of regular endurance training (Fehrenbach et al., 2000).

Table 2. 3: Summary Table of a sample of human PBMC and Leukocyte HSP72 response studies

Authors	Tissue	Measurement	Exercise Protocol	Results
Submaximal exercise				
Fehrenbach et al. (2000)	L	FC	Half marathon	~ HSP72
Shastry et al. (2002)	L	WB	60 mins running at 70% VO_{2peak}	↔ HSP72
Connolly et al. (2004)	PBMC	GA	30 min constant cycling at ~80% VO_{2max}	↑ HSP72 IP (2 fold) & 60 min post (2.4 fold)
Shin et al. (2004)	PBMC	NB & WB	60 mins running at 70% HRR	TR ↑ HSP72 at 30 min post (~130%) UTR ~ Hsp72
Chang et al. (2010)	PBMC	WB	60 mins running at 70% VO_{2peak}	↔ HSP72
Heat exertion stress				
Fehrenbach et al. (2001)	PBMC	RT-PCR & FC	2 x 60 min run at 90% IAT. NH 1st run at 18°C, 2nd run at 28°C & 50% RH. HH both runs at 28°C	↑ PBMC HSP72, 24 hr & 48 hr
Magalhaes et al. (2010)	PBMC	WB	HST 90 mins running at 50% VO_{2peak}	↑ PBMC HSP72 IP (~200%) and 1 hr post (~350%)
Marshall et al. (2007)	PBMC	QPCR & WB	2 hrs cycling at 38% VO_{2peak}	↑HSP72 (~275%)
Yamada et al. (2007)	PBMC	WB	100 min running at 56% VO_{2peak}	↑ HSP72 IP (161%) NS
Kuennen et al. (2011)	PBMC	WB	HTT 45 min running at 50% VO2peak in 47°C & 20% RH	↔ PBMC Hsp72

↑ (Increase), ↔ (No change), FC (Flow Cytometry), Fold (Fold change from pre exercise), GA (Gene array), HRR (Heart rate reserve), HSP72 (Heat shock protein 72 mRNA), IP (Immediately post), L (Leukocytes), NB (Northern blotting), NS (not significant), PBMC (Peripheral blood mononuclear cells), QPCR (Quantitative polymerase chain reaction), RT-PCR (Real-time polymerase chain reaction), WB (Western blotting), VO_{2peak} (Maximum oxygen update).

2.5.4 Heat Shock Proteins and Heat

Intracellular increase in HSP72 expression has been shown in reaction to exercise and heat stress in leukocytes (Fehrenbach et al., 2001; Fehrenbach et al., 2000). Ryan et al. (1991) was among the first to observe HSP72 in leukocytes changes during exercise in the heat in humans. It was reported that post exercise samples from participants with rectal temperature <40°C did not prevent further HSP synthesis when incubated at 31° or 41°C, suggesting that rectal temperatures >40°C is necessary to provide sufficient stress within the cells to elevate Hsp72 expression however, this is difficult to assess in a laboratory setting due to ethical guidelines. Studies have shown that endurance athletes can endure Tcore as high as 41.5°C without producing symptoms of EHI (Byrne et al., 2006). Additionally, within a triathlon it has been reported athletes who collapse were not more hyperthermic than some that didn't (Noakes, 2006). It has been suggested that these individuals may be more 'protected' from EHI on a cellular level, intracellular HSP72 may be involved in improving cellular thermotolerance. These findings could be applied to occupational pursuits such as the military to explain why some soldiers during training suffer from EHI while others do not.

Magalhaes et al. (2010) detailed an acute exercise induced increase in intracellular HSP72 expression at baseline after 6 days of heat acclimation, however, this conflicts with previous findings by Yamada et al. (2007) and Marshall et al. (2007). One possible explanation for this is $T_{core,}$ within Magalhaes et al. (2010) study average maximum T_{Rectal} reached was 39.2°C, while participants in the previous two studies average T_{Rectal} did not surpass 39°C. Marshall et al. (2007) reported a parallel between reduced thermoregulatory and cardiovascular strain with an attenuation in rise of PBMC HSP72 mRNA expression during exercise. These results indicate that HSP72 mRNA expression could be a reliable thermal stress biomarker not only for heat acclimation but also to gauge the effect of intervention strategies.

2.5.5 Heat Shock Proteins as Thermal Markers

Taking all previous findings into consideration it could be possible to use HSP72 expression in leukocytes as a molecular gauge of stress response to assess the effectiveness of intervention strategies. Limited research has investigated the influence of hydration (Hillman, 2013) and vitamin supplementation (Jackson et al., 2004) on HSP70 expression. Effects of hydration status on HSP72 during exercise has not been thoroughly investigated particularly in humans to date, it has been shown that dehydration causes impaired protein synthesis through blocking heat shock expression (Kurz et al., 1998). This suggests that hydration status is an important factor involved in cellular protection and thermotolerance via HSP72 expression. No research has used HSP72 leukocyte expression as a molecular stress biomarker for pre-cooling intervention strategies or a combination of hydration and pre-cooling on exercise in the heat.

2.6 Overall Summary

Overall it can be seen that the response of the human body to exercising in the heat involves complex integration of all the body's systems from cardiovascular to neuromuscular to cerebral function. It has generally been accepted that cerebral function mediates the response to alterations such as body temperature, through receiving and sending signals to central and peripheral receptors to inhibit/synthesis mechanisms to dissipate heat (Nybo, 2007). The primary focus of this thesis was to investigate the effect of intervention strategies to reduce thermoregulatory strain and improve exercise performance in the heat. Focusing on reducing the risk of EHI in athletes and military personnel and taking into consideration the time constraint which limits their ability to reduce thermal strain and dehydration during training and/or operation. With significant thermoregulatory and performance results reported for both glycerol-hyperhydration and pre-cooling intervention strategies (see table 2.1 and 2.2), a combination of the two may provide optimal benefit to exercise in the heat.

Limited research has investigated the interrelated effect of hydration and established pre-cooling strategies (Hasegawa et al., 2006; Ross et al., 2012). Hasegawa et al. (2006) research was limited as they combined water hyperhydration with pre-cooling, performance was significantly improved but the addition of a fluid retaining substance such as glycerol was not investigated. Ross et al. (2012) reported no change in performance after glycerol hyperhydration combined with ice slurry ingestion, however the control condition used water of the same volume but also at a cold temperature resulting in a substantial pre-cooling effect. Both previous studies used cycling for the exercise protocol; running is more applicable to a wider population for example occupational pursuits and recreational athletes. Additionally, it uses a greater number of muscles groups, high metabolic heat production from which creates higher strain on heat dissipation mechanisms making the intervention strategies employed central to delaying onset of fatigue.

Indeed, with glycerol hyperhydration's ability to delay the onset of dehydration through retention of fluid and therefore delaying sweating threshold and pre-cooling's mechanism to reduce T_{core} and in some cases T_{skin}, the combination could significantly improve exercise capacity and reduce thermal strain. It is also necessary to investigate effective pre-cooling methods which are logistically practical with ease of application. As detailed previously, there is limited but promising literature demonstrating acetaminophen's hypothermic mechanism of action when exercising in the heat (Mauger, Taylor, Harding, Wright, et al., 2013) It has not yet been determined how acetaminophen's hypothermic action works; whether T_{core} is reduced post ingestion or the rise in temperature is attenuated during the exercise.

HSP72 expression in leukocytes can give a molecular gauge of the cellular response to exercising in hot and humid environments and whether the intervention strategies employed attenuate its expression. Utilising HSP72 as a cellular stress biomarker in relation to pre-

cooling methods effect has not been previously researched. It will provide an insight into whether hydration and pre-cooling intervention strategies have an effect on not just physiological responses but also at a cellular level.

2.7 Research questions and experimental hypotheses

Experimental Chapter 1 - Molecular and performance effects of pre-cooling and hydration

1) To investigate the effect of combined hyperhydration and pre-cooling on cycling performance in hot and humid conditions

   - The combination of hyperhydration and pre-cooling improve time trial performance in the heat more than either strategy alone.

2) Secondly to investigate HSP72 mRNA expression as a thermal stress marker between conditions.

   - HSP72 mRNA expression will be attenuated greatest after the combined G-HH and CWI strategies.

Experimental Chapter 2- Glycerol hyperhydration effects on plasma volume

1) To investigate the peak % change in plasma volume after glycerol hyperhydration compared to water hyperhydration.

   - Glycerol hyperhydration peak plasma volume will be higher than water hyperhydration

2) Secondly, to observe the time course of plasma volume expansion suffices for after glycerol hyperhydration in sedentary state.

   - Plasma volume expansion will suffice for longer after glycerol hyperhydration compared to water hyperhydration

Experimental Chapter 3 – Molecular and Thermophysiological effects of acetaminophen as a pre-cooling agent

1) To compare the thermoregulatory effect of cold water immersion and ice slurry ingestion pre-cooling methods with acetaminophen on sub maximal exercise in extreme heat.

   - Cold water immersion will produce the greatest reduction in thermal strain, acetaminophen will have a pre-cooling effect on the thermoregulatory system reducing thermal strain when exercising in the heat.

2) Secondly to investigate HSP72 mRNA expression as a thermal stress marker between conditions exercise in extreme heat.

   - HSP72 mRNA expression will be attenuated the most after cold water immersion due to it having a significantly larger effect on cellular stress.

CHAPTER 3: General Methodology

This chapter illustrate the general procedures employed in the studies described in the following experimental chapters. Within the methods section of each experimental chapter, specific procedures will be outline and reference will be made back to appropriate sections with in the general methodology were necessary.

Ethical approval

Ethical approval was granted by the University of Bedfordshire, Department of Sport Science and Physical Activity Ethics in Human Research Committee.

3.1 Participants

The Participants for each experiment performed in this thesis were healthy males aged 20.2 ± 2 yrs. Participants were healthy and recreationally active university students. Before testing commenced informed consent in written format was obtained in the form of a PAR-Q (appendix b) and blood screening (appendix c). These documents helped to screen for any potential risk to the participants or the investigator(s) during the experimental period. Participants were made aware they could withdraw from the study at any time without any disadvantage or explanation. Participants were informed of the procedures and risks involved in par taking and had the chance to ask any questions, they were given information sheet (appendix d) detailing the purpose of the research. Throughout the entire experimental period, participants were monitored and in the event of participant discomfort testing would be terminated and always had constant supervision of the investigator and a first aider.

Anthropometric data

Body mass (kg) and height (cm) were used as subject descriptive data and were measured using Tanita scales (Tanita, BWB0800, Allied Weighing) and Stadiometer (Harpenden HAR-98.602).

Pre experimental diet and exercise standardisation

The following confounding variables were controlled for in order to minimise effect on the experimental variables in all experiments: participants refrain from consuming caffeine and alcohol 24 hours prior to testing. Additionally, participants abstained from smoking, glutamine, generic supplementation, thermal exposures, hypoxic exposures and hyperbaric exposures during the full experimental period (Taylor et al, 2010; Hillman et al, 2011). The afore mentioned control measures are in line with prior exercise and stress protein based research within the field Compliance for all the aforementioned potential confounding variables, if not excluded during subject recruitment, was monitored via a questionnaire administered before, during and post the extended 13-day study period and was 100 % in all subjects. Experimental conditions were completed at the same time of day to minimise the influence of circadian variations in basal HSP72 (Sandstroem et al. 2009).

3.2 Experimental trials

Ambient temperature and relative humidity were monitored continuously using a calibrated handheld hygrometer (Comark N006, Hertfordshire, UK) and via the thermometer linked to the chamber control panel. Temperature sensors were calibrated before all experimental trials.

3.3 Hydration Status Assessment

Prior to arriving at the laboratory in all experimental studies, participants were instructed to drink 500 ml of water 2 hr prior to exercise bouts, in accordance with the ACSM position stand (Sawka et al., 2007). Upon arrival to the laboratory hydration status of participants was assessed via urine osmolality and plasma volume (collected via capillary blood sample). If osmolality was <600 Osmo participants were deemed to be hydrated, >600 Osmo they were dehydrated. In such incidences participants were instructed to drink a further 500 ml of water and the study was only begun once participants reached the hydrated criteria. Additional time points for assessment of urine osmolality and plasma volume are highlighted in their respective chapters.

3.4 Thermoregulation Measurement

Rectal temperature

Rectal temperature (T_{re}) was measured continuously using a rectal probe (Henley's, 400h & 449IH, Henley's) inserted by the participant 10cm past the anal sphincter and connected to a temperature monitor (Libra Medical, ET402, Cranlea). If T_{rectal} reached or exceeded the safety limit of 39.5°C or 2°C above starting temperature they will immediately be removed from the environmental chamber as recommended by the institutional ethical board.

Skin temperature

Thermistors were attached to the pectoralis major (T1), triceps brachii (T2), rectus femoris (T3) and gastrocnemius (T4) (EUS-U-VS5-0, Grant Instruments, Cambridge, UK) on the right side of the body to measure skin temperature continuously via a monitor (Squirrel meter

logger, Grant Instruments, Cambridge, UK). Mean skin temperature was calculated using an equation:

$Tskin = [0.3(T1 + T2)] + [0.2(T3 + T4)]$ (Ramanathan, 1964).

Mean body temperature (Tb) was calculated using the following equation (Colin et al., 1971):

$Tb = (0.79 \times Tc) + (0.21 \times Ts)$

Heat storage (HS) was calculated using the following equation (Adams et al., 1992):

$$HS = (W \cdot m^{-2}) = 0.965 \times \frac{BM \times \Delta Tb}{Ad}$$

Heart rate and subjective measures

Heart rate was recorded continuously using a transmitter strapped, portable heart rate unit (Polar, FSI, Cranlea). Participants were asked to rate their perception of thermal sensation (TSI) on a scale of 0 – 8 (Young et al., 1987) and ratings of perceived exertion were recorded using Borg's 6 – 20 scale (Borg, 1982), these ratings were recorded at rest and every 5 minutes during experimental trials.

3.5 Blood collection

Blood lactate, glucose and plasma volume concentrations

Fingertip capillary blood samples were obtained from each participant at rest, and every 10 minutes during the experimental trials to determine blood lactate (Bla), blood glucose (BGlu) and plasma volume (PV) through haematocrit and haemoglobin concentrations. The sampling fingertip was cleaned with an alcohol swab and allowed to dry; the skin was then punctured with an automated lancet and samples were collected into heparinsed capillary tubes (Hawksley & Sons Ltd, UK), they were then centrifuged at 5,000 RPM for 4 min (Hawksley Micro Haematocrit centrifuge) and haematocrit levels were read from the Haematocrit reader

(Hawksley, UK). Further blood samples were collected in microcurvettes and measured for haemoglobin concentrations using a B-Haemoglobin photometer (Hb 201^{+}, Hemocue Ltd, UK). Changes in plasma volume (%ΔPV) is then estimated from haemoglobin (Hb) and haematocrit (Hct) using the following equation (Dill & Costill, 1974):

$\%\Delta PV = [(Hb_{preex}/Hb_{postex}) \times [(100 - Hct_{postex})/(100 - Hct_{preex})] - 1] \times 100.$

$$\Delta PV = \left[\left(100 - \frac{Hb_b}{Hb_a}\right) - \times \frac{1-(Hct_a-100)}{1-(Hct_{b-100})}\right] - 100$$

Where ΔPV is percent change of PV, subscript b is prior to exercise and subscript a is post exercise.

Venous blood sample

Figure 3. 1: illustration of a venous blood sample being collected.

In experiments 1 and 3 venous blood samples were drawn by standard venepuncture from an antecubital vein using a 21 gauge needle. Blood was inverted into a heparin vacuette tube

(Vacuette®, Greiner Bio-one, UK). Blood samples were collected at the following time points: rest, pre exercise and post exercise (see figure 3.1).

3.6 Molecular physiology measurements and apparatus

Blood Samples (Leukocyte & RNA isolation)

Venous blood was obtained from the antecubital vein into a 6ml EDTA tube (K2, Grenier Bio One). Using an adaptation of a previously validated method (Hillman et al., 2011; Peart et al., 2011; Sandstroem et al., 2009; Taylor et al 2012; Taylor et al., 2011), 500µl of venous blood was pipetted into 10ml of 1 in 10 red blood cell lysis solution (10X red blood Cell Lysis Solution, Miltenyi Biotech, UK). Samples were incubated for 15 min at room temperature and then isolated via centrifugation at 400G for 5 min and washed twice in 2ml PBS at 400G for 5 min. RNA was then extracted from the leukocytes via a previously validated method (Chomczynski & Sacchi, 1987). Briefly 200 µl of TRI-reagent (Sigma Aldirch, Poole, Dorset) was added and samples were incubated on ice for 10 min. 40µl chloroform (Sigma Aldirch, Poole, Dorset) was added and each sample was vortexed for 15 s prior to the centrifugation of samples at 17,000 G for 15 min. The aqueous layer containing the RNA was then removed and placed in a separate 1.5 ml centrifuge tube to which an equal amount (~100µl) of isopropanol (Sigma Aldirch, Poole, Dorset) was added. Samples were then vortexed for 15 s, placed on ice for 15 min and centrifuged for 15 min at 17,000 G. The supernatant was then discarded and the RNA pellet was washed with 100 µl 75% ethanol (Sigma Aldrich, Poole, Dorset) and centrifuged for 8 mins at 5,400 G. The ethanol was then discarded and the samples were centrifuged for a further 30 s at 17,000 G to spin any remaining supernatant to the bottom of the tube for removal via pipetting. The RNA pellet was air dried for 10 min prior to being resuspended in 50µl of RNA storage solution (The RNA storage Solution, Ambion). Quantity was determined at an optical density of 260 nm

while quality was determined via the 260/ 280 and 260/ 230 ratios using a nanodrop spectrophotometer (Nanodrop 2000c Thermo Scientific).

One step reverse transcription quantitative polymerase chain reaction (RT-QPCR)

Primers (see table 3.1) were designed using primer design software (Primer Quest and Oligoanalyzer - Integrated DNA technologies). During primer design sequence homology searches were performed against the Genbank database to ensure the primers matched the gene of interest. Primers were designed to span exon-intron boundaries and avoided three or more GC bases within the last 5 bases at the 3' end of primer to avoid non specific binding. Further searches were performed to ensure primers did not contain secondary structures and inter or intra molecular interactions (hairpins, self-dimer and cross dimers) which can inhibit product amplification. Primer sets were validated via conventional PCR and agarose gel electrophoresis to ensure the correct product size was amplified. B_2 – microglobulin was used as the housekeeping gene as its expression is unregulated and constant in experimental conditions (Jemiolo & Trappe, 2004). HSP relative mRNA expression (HSP) 72 was then quantified using RT-QPCR. 20 µl reactions containing 10 µl SYBR-Green RT-PCR Mastermix (Quantifast SYBRgreen Kit, Qiagen), 0.15 µl forward primer, 0.15 µl reverse primer, 0.2 µl reverse transcription mix (Quantifast RT Mix, Qiagen) and 9.5 µl sample (70 ng RNA/µl) were prepared using the Qiagility automated pipetting system (Qiagen, Crawley, UK). Each PCR reaction was amplified in a thermal cycler (Rotorgene Q, Qiagen) for 40 cycles using a denaturation step lasting 10s at 95°C and a primer annealing and extension stage lasting 30s at 60°C. Fluoresecence was measured following each cycle as a result of the incorporation of SYBR green dye into the amplified PCR product. Melt curves (50 to 95°C; Ramp protocol 5s stages) were analysed for each reaction to ensure only the single gene of interest was amplified.

Table 3. 1: Real-time qPCR primer sequences.

Gene	Primer Sequence (5'-3')	Ref. Sequence Number	Amplicon Length (bp)	GC% Content
HSP72	**F**: CGCAACGTGCTCATCTTTGA	NM_005345.5	198	50
	R: TCGCTTGTTCTGGCT GATGT			50
β2-Microglobulin	**F:** CCGTGTGAACCATGTGACT	NM_004048.2	91	52.63
	R: TGCGGCATCTTCAAACCT			50

The relative quantification of mRNA expression for each sample was assessed by determining the ratio between the C_T value of the target mRNA and the C_T values for β2-Microglobulin (β2-M) (See equation 1). Fold change in relative mRNA expression was calculated using the $2^{-\Delta\Delta CT}$ method (See equation 1) (Schmittgen, 2008).

Equation: Delta Delta Ct ($\Delta\Delta C_T$) (Schmittgen, 2008)

Equation 1: ΔC_T = Mean C_T (Gene of interest, experimental sample) – Mean C_T (Housekeeping gene, experimental sample).

Equation 2: ΔC_T = Mean C_T (Gene of interest, calibrator) – Mean C_T (Housekeeping gene, calibrator).

Equation 3: $\Delta\Delta C_T = \Delta C_T$ of equation 1 – ΔC_T of equation 2.

Equation 4: $2^{-\Delta\Delta CT}$ (gives a normalised expression ratio).

The mean C_T is the average C_T of a gene at a specific time point in duplicate. The calibrator is the sample which was given a value of 1.0 fold and which all other time points are expressed relative to. The experimental sample is any time point or condition other than the calibrator.

3.7 Statistical analysis

All data are presented as mean ± SD. Analyses were completed using the statistical software package IBM SPSS statistics version 19.1 (SPSS Inc, Chicago, IL, USA) and GraphPad Prism (GraphPad software Inc, California, USA). Statistical assumptions were checked using conventional graphic methods (Grafen & Hails, 2002) and were deemed plausible in all instances. Prior to any inferential statistical analysis, descriptive statistics were generated to dispersion (SD, minimum, maximum) and central tendency (mean, median). Statistical analysis was completed using linear mixed models were used to determine if there were any differences in the dependent variables between the conditions over time during across trials. This type of analysis was preferred as it allows for missing data and can specify covariate structures for repeated measures data. First fixed and random factors for the linear mixed model were fit for each dependent variable and the main effects for trial, group and the interaction effect (condition x time) were analysed by plotting the mean values. The most appropriate model was chosen using the likelihood ratio test. This method uses the $\chi 2$ critical test statistic to decide which model is the best fit based on the change in the -2 restricted log likelihood of two models. Where a significant F ratio was observed post hoc comparisons with Sidak-adjusted p values were used to identify which pairs of means were different. Two tailed statistical significance was accepted as $p < 0.05$. 95% confidence intervals (CI) were also reported. Finally, Cohen's effect sizes (ES) for independent means were calculated utilising the formula outlined by Cohen (1992):

$$d = \frac{\mu_a - \mu_b}{\sigma}$$

CHAPTER 4: Experiment 1- the combined effect of hyperhydration and pre-cooling on endurance cycling performance in hot and humid conditions.

4.1 Introduction

Endurance exercise and occupational pursuits (e.g. military and disaster emergency workers) challenge the human body via circulatory and thermoregulatory strain (Maughan et al, 2012). The environments (e.g. extremely hot and humid) such activities are often performed in, can further compound this physiological strain (Gonzalez-Alonso et al., 2008; Gonzalez-Alonso et al., 1999). In order to attenuate this heat-exercise mediated performance decrement various interventions have been employed, for example external pre-cooling (Duffield et al., 2010), internal pre-cooling (Siegel et al., 2010) and hyperhydration (Anderson et al., 2001) with varying degrees of efficacy.

A well-established intervention to address heat-exercise mediated decrements in performance is pre-cooling. This reduces pre-exercise T_{rectal} and skin temperature (T_{skin}), which serves to increase latent body heat capacity, thus improving the efficiency of heat dissipation mechanisms and delaying the onset of hyperthermia related fatigue (Olschewski et al., 1988). Some pre-cooling methods with substantial ergogenic effect can take long durations to perform (e.g. up to 2 h for cold air exposure), which can be impractical and logistically challenging in a field setting. CWI however, cools a large portion of the body in comparison to methods such as ice vests and ice packs; and can decrease T_{rectal} and T_{skin} for longer durations, by a greater magnitude and more rapidly (Duffield et al., 2003).

A plethora of research has also demonstrated that maintaining hydration during exercise in the heat can lead to improvements in overall performance (Sawka et al., 1992; Goulet et al.,

2006; Murray et al., 1991; Latzka et al., 1997). It is impractical or in some cases unfeasible to hydrate during some forms of exercise and therefore, it may be advantageous to increase hydration to a level of hyperhydration prior to exercise (Goulet et al., 2006). Hyperhydration can easily be achieved by a combination of glycerol and water (Freund et al., 1995; Hitchins et al., 1999; Latzka et al., 2000), which may serve to delay the onset of dehydration, enhance sweat rates and thus potentially augment heat dissipation mechanisms. It is currently unknown which of these interventions (i.e. pre-cooling or hyperhydration), or indeed whether a combination of both, may favourably alter thermoregulatory responses (T_{rectal} and T_{skin}) more, delay the onset of dehydration, and maximise/maintain optimal sweat rates. Subsequently leading to improved endurance performance in hot and humid environments.

The thermoregulatory challenge of exercise in the heat can be observed through physiological responses such as T_{rectal} and T_{skin} but also at a molecular level, by observing molecular and cellular biomarkers of heat and exercise tolerance/stress. Exercise and heat stress, in isolation and combination, causes cells to rapidly produce a highly conserved family of stress proteins, more commonly termed heat shock proteins (HSP) Differential HSP72 gene expression, pre to post exercise between conditions (pre-cooling and/or hyperhydration combinations), could provide insightful mechanistic evidence of heat-exercise induced disturbances to homeostasis (Maloyan et al., 1999). It is of interest if any observed ergogenic effects from the interventions (pre-cooling and/or hyperhydration combinations); influence the magnitude of HSP gene expression pre to post exercise.

Aim

The primary purpose of this study was to investigate hyperhydration (glycerol hyperhydration) and pre-cooling (CWI) strategies; individually and in combination, on cycling time trial (TT) completion time in hot and humid conditions. It was hypothesised that,

firstly: 10 mile (16.1km) TT completion time would be quickest after the combined strategies of pre-cooling and hyperhydration (PC+HH), in excess of pre-cooling alone (PC) or hyperhydration alone (HH). Secondly, HSP72 mRNA relative expression (HSP72) would be attenuated the most after PC+HH.

4.2 Methods

Subjects

Five recreationally active, healthy males (mean ± SD: age 20 ± 1 y, height 179 ± 4 cm, weight 74.7 ± 5.8 kg and $\dot{V}O_{2peak}$ 45 ± 2 mL·kg $^{-1}$·min^{-1}) participated in this study.

Preliminary Measurements

Prior to completing conditions in experiment one, participants underwent a $\dot{V}O_{2max}$(maximum oxygen uptake) test on a Lode cycle ergometer (Lode, Excalibur Sport, Cranlea, Birmingham, UK) using a standardised incremental protocol (Mauger et al., 2009). Following a 5 min self-paced warm-up, participants cycled at an initial intensity of 150 W with 20 W increments every 2 min until volitional exhaustion. Expired gas was measured continuously throughout the test using indirect calorimetry (Cortex, Metalyser 3B, Cranlea, Leipzig, Germany) to obtain minute ventilation, carbon dioxide production and oxygen uptake. Heart rate (HR) was measured throughout the test. Blood samples were obtained at the end of each 2 min stage using standardised Heparin coated Microhaematocrit capillary tubes (Marienfeld, Germany) and analysed for blood lactate (B[La]; Analox LM5/P-GL5, London, UK). Following a standardised rest period (40 min) after the VO2max test, participants completed a familiarisation 10 mile (16.1 km) TT on a Computrainer (RacerMate, Computrainer Lab R985 192, SCFI Fitness and Health, Seattle, USA) in

temperate conditions (18 °C, 35% RH). Participants were blinded to the distance covered and time elapsed.

Experimental Design

The remaining four visits consisted of exercise trials either with Glycerol hyperhydration (HH) or pre-cooling (PC), both glycerol hyperhydration and pre-cooling (PC+HH) or neither (C) see figure 4.1. The exercise protocol used was a fixed distance self-paced time trial cycle for 10 miles (Mauger et al., 2010), participants were asked to complete the time trial in as quick a time as possible to reflect authentic cycling performance. All subjects completed all time trials. The experiment was a single blinded (in respect to the glycerol treatment) repeated measure, cross over design. See figure 4.2 for experimental schematic detailing the layout of the experimental trial and the parameters measured. This study was conducted in an environmental chamber (WatFlow control system, TISS, custom built, Hampshire, UK) averaging at 30 ± 0.2 °C and 50 3.4 % relative humidity (RH).

See section 1.2 for hydration status necessities of participants prior to exercise. Subsequently participants consumed either a glycerol (in HH and HH+PC conditions) or placebo solution (in C and PC conditions) over a 90 min period with instructions to drink it at a steady and even rate over the allotted time. The glycerol solution consisted of 1.2 g · kg of BM glycerol mixed in 26 ml·kg of BM of water (Goulet, 2010). The placebo solution entailed aspartame-flavoured water 0.156 g/kg of BM of equal volume to the glycerol drink (Goulet et al., 2006). No additional fluid was consumed during the time trial (TT).

Upon completion of the fluid ingestion participants rested in a supine position for 10 minutes prior to blood sample collection (described in section 3.5). Please see general methods,

section 3.4 for information regarding the attachment of temperature and heart rate equipment. In the pre-cooling conditions participants were instructed to enter the inflatable ice bath (inflatable ice bath, Gold White Fitness, Ramrugby.co.uk) submerged up to their waist with the water at a temperature of 14 ± 2˚c. The water immersion lasted 20 minutes (Duffield et al., 2010). T_{rectal} and T_{skin} were monitored continuously throughout. After the pre-cooling, participants were towel dried and escorted to the environmental chamber (<5 min) to commence the 5 min self-paced warm up before beginning the 10 mile (16.1 km) TT.

See figure 4.2 for timing of parameter measurements such as T_{rectal}, RPE, TSI and T_{skin}. Following completion of the TT participants were removed from the environmental chamber and post exercise BM, BLa, BGlu, Hct, Hb, T_{re}, T_{sk} urine osmolality and venous blood sample were obtained as previously described.

Statistical Analysis

Statistical analysis was completed using linear mixed models for repeated measures (SPSS 19.1, Chicago, IL, USA) to analyse TT completion time, BM and BHC. Condition * distance was used to examine changes in T_{rectal}, T_{skin}, HR, RPE and TSI, B[La], B[Glu], PV and HSP72 between all conditions. Ninety five percent confidence intervals (95% CI) and effect sizes were also presented. Two tailed significance was accepted as $p < 0.05$.

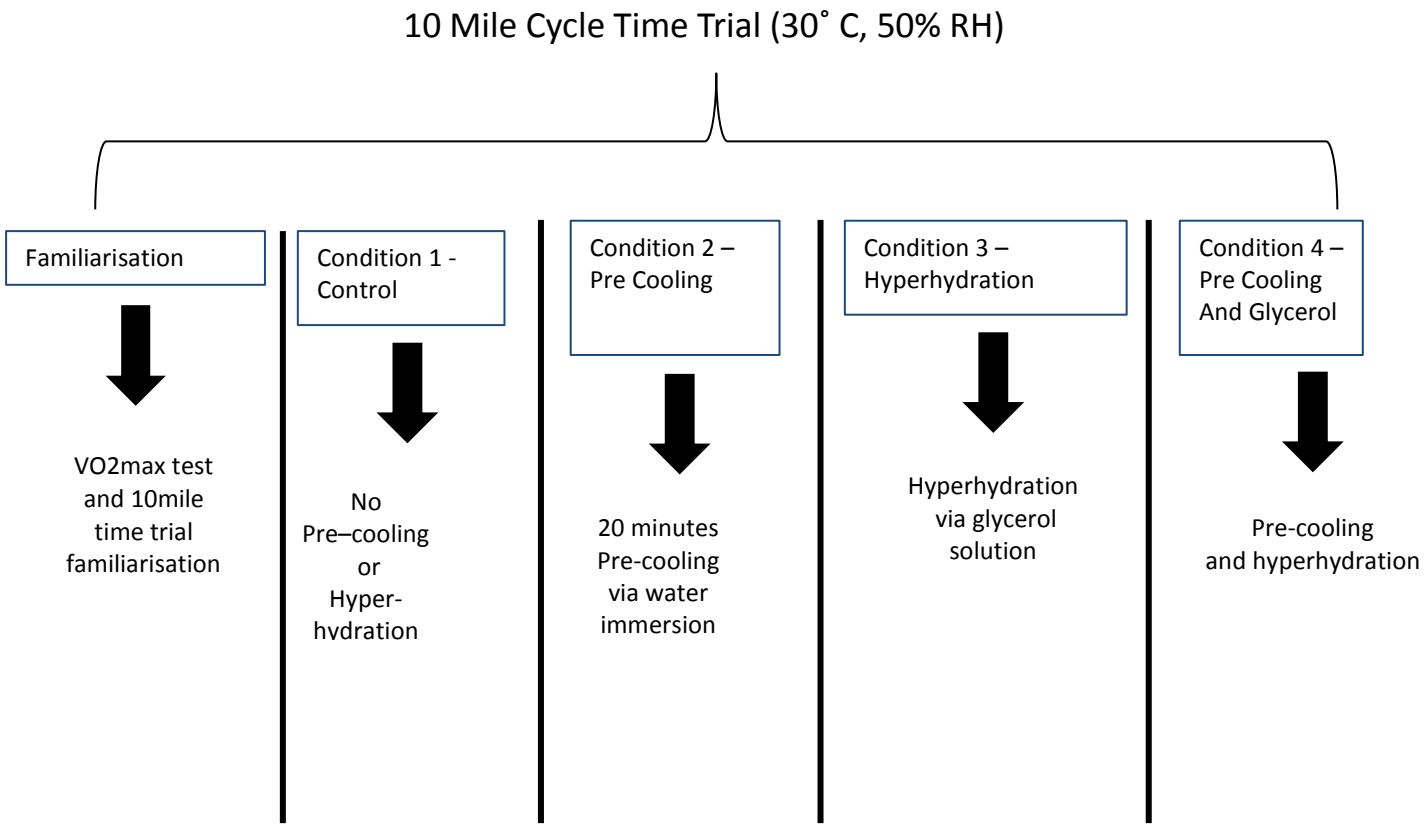


Figure 4. 1: Experiment chapter one overview schematic

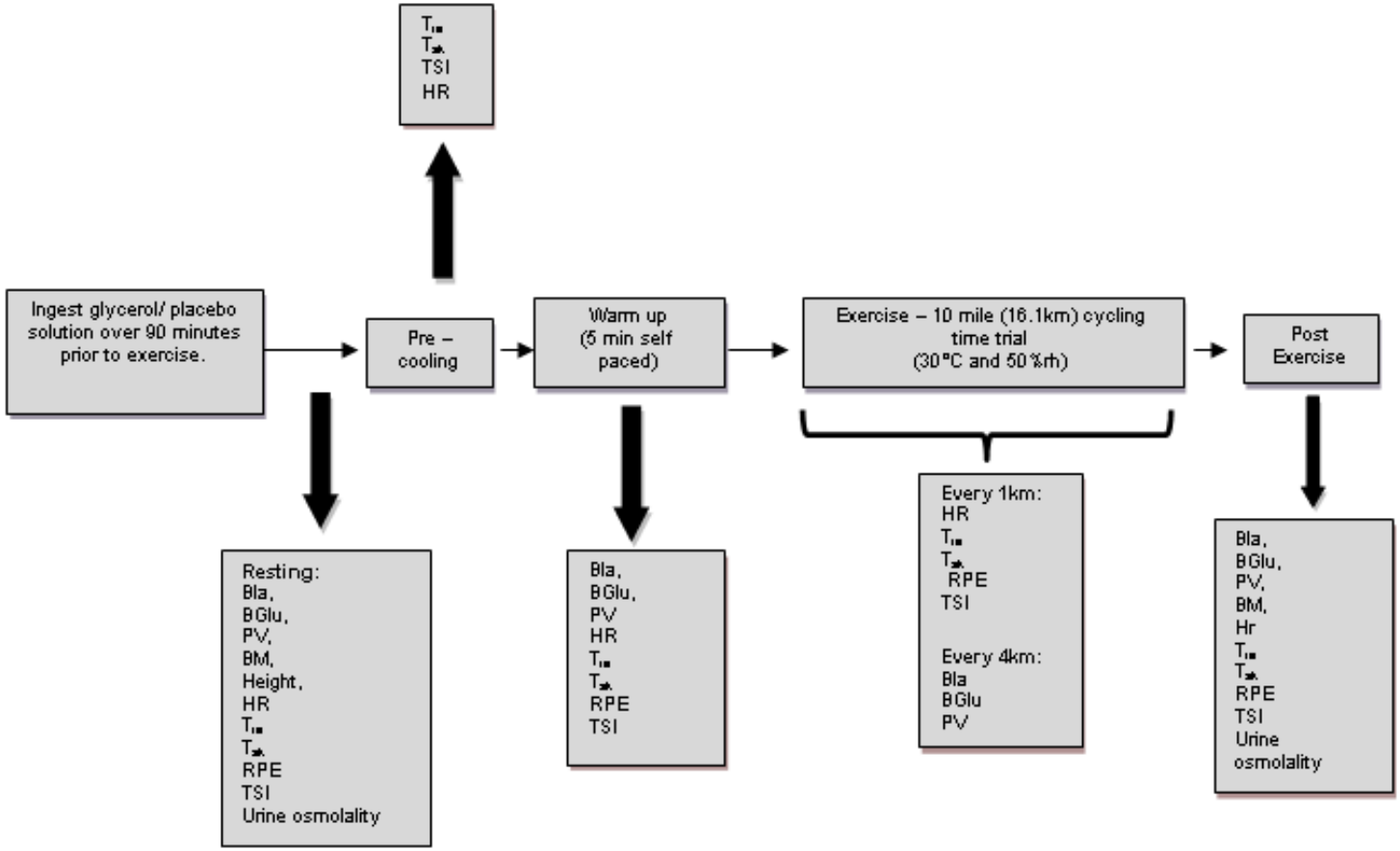


Figure 4. 2: Experimental chapter one protocol schematic

4.3 Results

Completion time

There was a significant interaction effect for 10 mile TT ($F_{3, 12}$ = 4.52 p = 0.03) (figure 4.3). On average completion time was 6% faster in PC compared to C (p = 0.03, 95% CI = 15 to 210 s) producing a moderate ES (0.78) and 4% faster than HH (p = 0.02, 95% CI = 21 to 132 s) also producing a moderate ES (0.69).

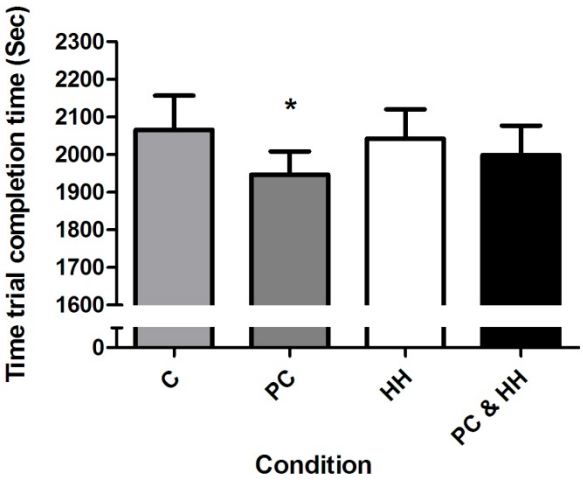


Figure 4. 3: Presents mean (n = 5) ± SD data for time to completion of 10 mile (16.1km) in each condition. *significantly shorter time to completion after PC compared to C (p < 0.05).

Thermoregulatory responses

There was significant interaction effect for T_{rectal} over the 10 mile TT ($F_{7.7,\ 30.9}$ = 3.75 p = 0.004; Figure 4.4). From the onset of exercise T_{rectal} was lower for PC + HH compared to HH (p = 0.04, 95% CI = 0.04 to 1.7 °C) producing a small ES (0.43). The PC conditions (PC, PC+HH) continued to have lower T_{re} than C or HH throughout the 10 mile TT. By completion of exercise PC T_{rectal} was on average 1°C lower than HH (p = 0.01, 95% CI = to 0.4 to 1.6 °C) producing a very large ES (2) and PC+HH final T_{rectal} was on average 0.9°C lower than HH (p = 0.02, 95% CI = 0.2 to 1.6°C) producing a large ES (1.29).

There was significant interaction effect for T_{skin} during the 10 mile TT ($F_{2,\ 8.2}$ = 7.34, p = 0.01; Figure 4.5). At the commencement of the TT protocol T_{skin} was 10.3°C lower in PC+HH compared to HH (p = 0.001, 95% CI = 3 to 9.1°C) producing a very large ES (8.01), 9.3°C lower than C (p = 0.004, 95% CI = 2.1 to 9.5 °C) Large ES (7.1) and PC was 9.1°C lower than HH (p = 0.007, 95% CI = 2.1 to 9.0 °C) very large ES (8.6) and 8.1°C lower than C (p = 0.01, 95% CI = 1.5 to 8.2°C) very large ES (11.01). PC and PC+HH T_{skin} remained significantly lower than C and HH until 12 km into the time trial; after which only PC+HH was significantly cooler than HH (p = 0.03, 95% CI = 0.3 to 3.3°C). By the end of the exercise there was no significant difference between conditions.

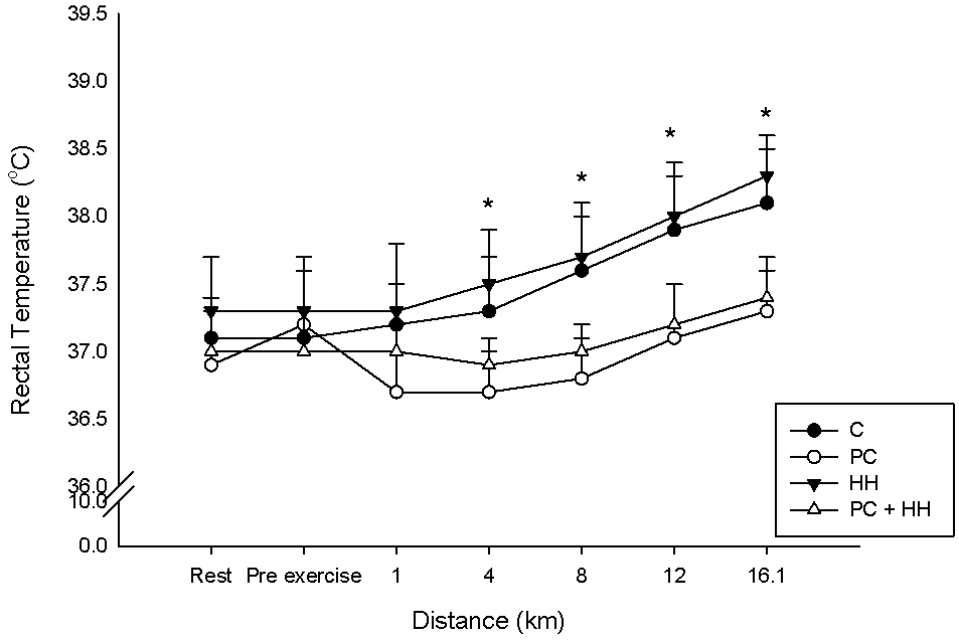


Figure 4. 4: Presents mean (n = 5) ± SD data for rectal temperature during the pre-cooling period and the 10 mile TT in all conditions. * Significantly lower rectal temperature after PC and PC+HH compared to HH and C ($p < 0.05$).

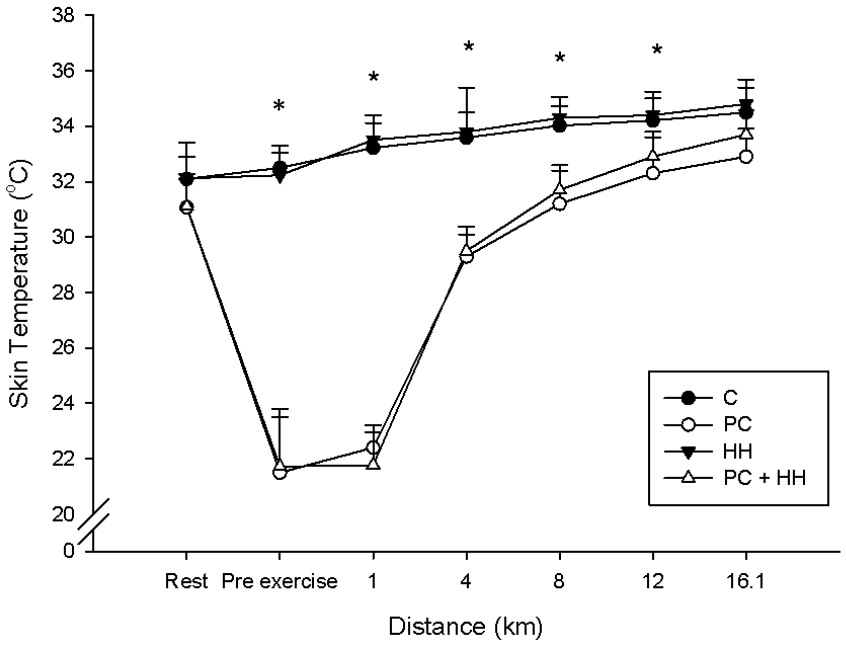


Figure 4. 5: Presents mean (n = 5) ± SD data for skin temperature during the pre-cooling period and 10 mile TT in all conditions. * Significantly lower skin temperature after PC and PC+HH than HH and C ($p < 0.05$).

Body heat content

There was a significant interaction effect for change in BHC between conditions ($F_{3,16}$ = 10.29, p < 0.001; figure 4.6). PC BHC was significantly lower on average than C (p = 0.001, 95% CI = 23 to 45 KJ·°C^{-1}·kg^{-1}) and HH (p = 0.03, 95% CI = 9 to 75 KJ·°C^{-1}·kg^{-1}). PC+HH BHC was also significantly lower than C (p = 0.01 95% CI = 14 to 57 KJ·°C^{-1}·kg^{-1}) and HH (p = 0.04, 95% CI = 5 to 82 KJ·°C^{-1}·kg^{-1}).

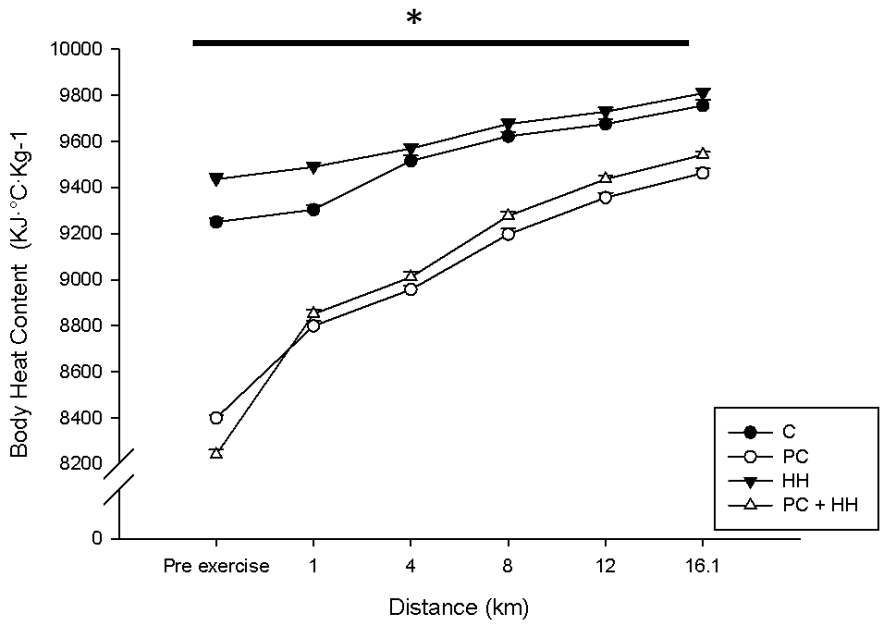


Figure 4. 6: Presents mean (n = 5) ± SD date for body heat content (BHC) during the 10 mile TT in all conditions. * Body heat content significantly lower throughout TT after PC and PC+HH compared to HH and C (P < 0.05). Error bars omitted for clarity.

Cardiovascular responses

There was no significant interaction effect for HR between conditions ($F_{4, 17}$ = 1.3 p = 0.31; figure 4.7).

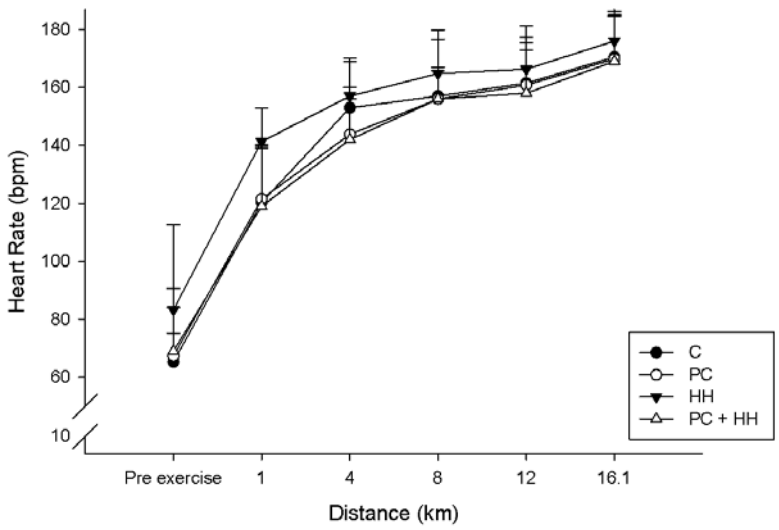


Figure 4. 7: Presents mean (n = 5) ± SD for heat rate (HR) during 10 mile TT in all conditions.

Hydration

There was no significant interaction effect for weight loss between conditions ($F_{3,16}$ = 0.71, p = 0.55) or urine osmolality between conditions ($F_{3,16}$ = 1.15 p = 0.35; Table 4.1). In all conditions weight decreased as would be expected when exercising in the heat due to sweat loss. There was also no significant interaction effect for PV during the 10 mile TT ($F_{3, 9}$ = 1.2 p = 0.45).

Blood measurements & subjective responses

There was no significant interaction effect for B[La] ($F_{12, 48}$ = 0.81 p = 0.63), B[Glu] ($F_{6,24}$ = 0.66 p = 0.68) or RPE ($F_{8.3, 33.4}$ = 1.14 p = 0.36) between conditions. There was a significant interaction effect for TSI during the time trial ($F_{8, 23}$ = 15.3 p < 0.001), with C demonstrating significantly higher ratings compared to PC (p = 0.04, 95% CI = 0.2 to 5.8) and PC+HH (p

=0.02, 95% CI = 0.8 to 5) at commencement of the exercise. After the 10 mile TT began there was no further significant interaction effect.

Table 4. 1: Represents the urine, haemodynamic and fluid changes for all trials.

	C		PC		HH		PC + HH	
	pre	**post**	**pre**	**post**	**pre**	**post**	**pre**	**post**
UOsm	126 ± 240	111 ± 61.1	180 ± 223	298 ± 201	176 ± 80.3	397 ± 174	218 ± 187	344 ± 118
BML	- -	0.92 ± 0.3	--	0.7 ± 0.23	--	0.92 ± 0.21	--	1.02 ± 0.11
%ΔPV	2.5 ± 2.51	2.4 ± 2.07	-2.9 ± 3.17	-0.1 ± 4.12	-1.4 ± 2.69	-0.4 ± 2.38	-2.3 ± 4.77	-2.4 ± 4.65

There was no significant effect for UOsm (urine osmolality) prior to the exercise protocol between conditions ($F_{3,16} = 1.15$ $p > 0.05$). There was no significant effect for body mass (BML) ($F_{3,16} = 0.71$, $p > 0.05$) and %ΔPV (percent change in plasma volume) ($F_{3,9} = 1.2$, $p = 0.45$) from pre to post exercise trial between conditions.

HSP72

There was no significant interaction effect for HSP72 gene expression across the four conditions ($F_{3\ 24} = 1.42$, $p = 0.26$; figure 4.8).

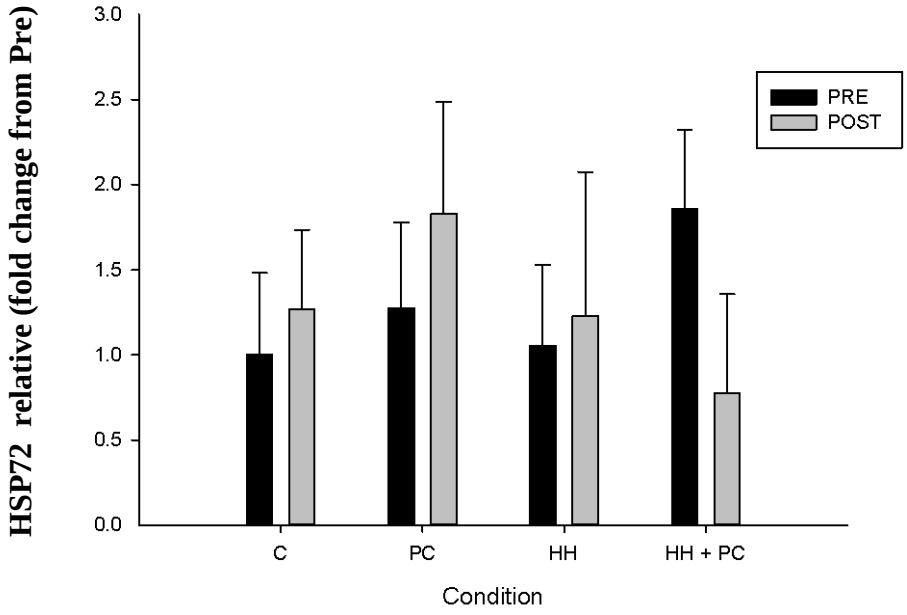


Figure 4. 8 : Presents mean (n = 5) ± SD data for Leukocyte HSP72 relative gene expression during the trial in all conditions.

4.4 Discussion

The purpose of this investigation was to examine the individual and combined effects of hyperhydration and pre-cooling as interventional strategies to improve cycling endurance performance in hot and humid environmental conditions (30°C & 50% RH). Firstly, it was found that pre-cooling alone or in combination with hyperhydration significantly reduced thermoregulatory strain (see Figure 4.4 and 4.5) and BHC in comparison to C and H. PC also improved completion time by 6% compared to C ($p = 0.03$, 95% CI = -15 to -210 s; Figure 4.3). These results suggest HH+PC is effective at reducing thermally induced fatigue when compared to C and HH, however, results suggest hyperhydration had no additional benefit to pre-cooling alone. Current literature consensus demonstrates a robust ergogenic effect of pre-cooling for cycling (Vaile et al., 2008), running (Siegel et al., 2010) and team sport performance (Drust et al., 2000) with the data from the present study contributing further to this growing body of literature. However, the experimental hypothesis that the combination of HH+PC would provide greater ergogenic response than PC alone cannot be accepted.

Pre-cooling

The findings of this study support CWI as an effective pre exercise intervention strategy; enhancing performance, reducing BHC and perceived thermal stress. The principal effect of pre-cooling was reduced T_{rectal} and T_{skin} pre exercise and attenuation of their rise during the 10 mile (16.1 km) TT (Figure 4.4 and 4.5). In the present study, T_{rectal} throughout the TT in pre-cooling conditions (PC and PC+HH) was lower than the conditions without (C and HH) and had the smallest change from commencement to completion of the exercise (0.1 ± 0.3 °C; 0.4 ± 0.2°C), compared with C (1 ±0.2°C) and HH (1 ± 0.3 °C; Figure 4.3). This differs from results observed using an ice vest to pre-cool (Quod et al., 2008) which reported no

significant change in T_{rectal} throughout the 40 min cycling TT compared to control, thus supporting the argument that CWI is a more effective method of reducing thermal stress. This positive thermoregulatory response to CWI was further observed in T_{skin}; pre-cooling conditions (PC and PC+HH) reduced starting T_{skin} by 10.3 ± 1 °C compared with the conditions without (C: 31.2 ±1.8°C; HH: 37.3 ± 0.4°C), showing similar results to cold air exposure without the cold stress response of shivering (Lee. et al., 1995). T_{skin} rose at a quicker rate during the 10 mile (16.1km) TT after CWI (PC and PC+HH) but the T_{skin} compared to C and HH was still significantly lower until the last 4 km of the TT.

The observed thermal responses to pre-cooling in the current study are predominately responsible for the reduced BHC during the TT (Marino, 2002). Previous research has shown that pre-cooling through a variety of methods; cold air, cold water and ice vests (Cotter et al., 2001; Lee. et al., 1995) significantly reduces BHC before the exercise commences. This supports results observed in the present study; after the CWI in the PC (8399 ± 65. 9 $KJ \cdot °C^{-1} \cdot kg^{-1}$) and PC+HH (8239 ± 77.1 $KJ \cdot °C^{-1} \cdot kg^{-1}$) BHC was significantly lower than HH (9435 ± 77.5 $KJ \cdot °C^{-1} \cdot kg^{-1}$) and C (9249 ± 32.6 $KJ \cdot °C^{-1} \cdot kg^{-1}$) continuing to completion of the TT. This indicates a greater rate of heat dissipation during the exercise, therefore delaying body thermal strain (Lee. et al., 1995).

CWI in recent literature has become the most common form of pre-cooling (Booth et al., 1997; Castle et al., 2006; Hasegawa et al., 2006; Kay et al., 1999). Marino (2002) stated that CWI is the most effective method for improving endurance performance and is highly advantageous for exercise in the heat. This is consistent with the results found in the present study with a significant reduction in T_{rectal}, T_{skin} and time to completion. However, defining guidelines for the application of pre-cooling is challenging due to the heterogeneous nature of methodologies used in previous research. Such studies utilised protocols of varying durations

and temperatures, ranging from 23 °C for 60 min (Booth et al., 1997) to 12 °C for 15 min (Schniepp et al., 2002). The positive physiological responses to the present study's protocol of 12 ± 2 °C for 20 min, indicates that shorter durations can elicit significant results. It is also essential that the pre-cooling method has ecological validity and is applicable to a field setting; CWI presents logistical challenges such as lack of facilities and time constraints. Recent research has investigated internal pre-cooling through cold water (Lee & Shirreffs, 2007) and ice slurry ingestion (Siegel et al., 2010), such methods are potentially more practical and easily accessible in a field setting; increasing ecological validity. Additionally, the novel use of acetaminophen as a reducer of thermoregulatory strain has recently been investigated (Mauger, Taylor, Harding, Wright, et al., 2013), a pharmacological agent would result in high ecological validity but more research need to be done to validate it as an effective pre-cooling method with significant ergogenic effect.

Hyperhydration

A reduction in exercise performance can occur through the interaction of decline in PV with increased skin blood flow, sweat rate, HR and RPE (Van Rosendal et al., 2010). The combination of these adverse effects makes it important to delay the onset of dehydration through a pre-exercise hydration strategy. Hyperhydration during exercise can be primarily indicated by expansion in PV. Murray et al (1991) looked at the effect of glycerol ingestion on performance of 90 min of cycling in 30°C and 40% RH, they demonstrated that PV significantly decreased in the first 30 min of exercise by 7-10%. The present study however, found there to be no added benefits of hyperhydration via glycerol than through water alone (placebo). There was no significant change in urine osmolality, weight loss or PV between hyperhydration conditions (HH and PC + HH) and those without (PC and C). One possible explanation for the inefficiency of glycerol to hyperhydrate, is the influence of the CWI. CWI

significantly increases dieresis which may alter glycerol's ability to induce osmotic gradients and retain water in the interstitial spaces of the body (Arnall & Goforth, 1993; Nelson et al., 2007). Observations of PV post pre-cooling could illustrate any reductions potentially offsetting PV expansion caused by glycerol hyperhydration. Hasegawa et al (2006) examined the combined effect of water hyperhydration and PC via water immersion on time to exhaustion (80% VO_{2max}), their results concurred that the combination of pre-cooling and water hyperhydration resulted in the longest time to exhaustion compared to PC alone, differing from the conclusion of the present study. This may be due to the warmer water temperature (25°C), compared to the present study (12°C). Colder water produces a more powerful stimulus for diuresis (Deuster et al., 1989) which could explain the lack of ergogenic effect from glycerol hyperhydration within the present study.

HSP72

Leukocyte HSP72 mRNA expression did not change from pre to post exercise. Although pre-cooling conditions (PC, PC+HH) decreased thermal stress and improved exercise performance there was also no change in leukocyte HSP72 compared to the other conditions (C, HH). This could suggest that the intervention strategy only reduced thermal strain at a physiological level with little to no effect on the cellular biomarkers of heat stress. However, due to there being no significant HSP72 increase post exercise compared to pre in any condition a more likely explanation would be the exercise mode employed not eliciting a sufficient cellular stress to stimulate a significant elevation in HSP72. It has been reported that HSP72 expression can be affected by the exercise mode employed (Yamada et al. 2008) with a self-paced TT as used in the present study, allowing the body to adopt a pacing strategy (Mauger et al. 2009) in response to rising levels of stressors. This in turn reduces the cellular stress compared to a time to exhaustion protocol; no research however, has looked at

the HSP72 response to TT protocols to date. Another possible explanation is that HSP72 expression is Tcore dependent. Selkirk et al (2009) showed that intracellular monocyte HSP72 % expression increased significantly once Trectal surpassed 38.5°C when exercising in 40°C. Referring to the Trectal data from the present study peak Trectal did not surpass 38.5°C in any of the conditions C (38.09 ± 0.08°C), PC (37.3 ± 0.12°C), HH (38.2 ± 0.11°C) PC+HH (37.45 ± 0.21°C) it is likely that the required endogenous criteria for increasing HSP72 was not reached. This suggests that a longer duration and higher fixed intensity exercise protocol could provide sufficient cellular stress to alter HSP72 kinetics and therefore, enable differentiation between intervention strategies in the current study.

Limitations

A limitation of the experimental study 1 is its small sample size (n = 5); recruitment of larger participant numbers would be necessary in future to validate results. Nevertheless, although the sample size is small, there was a large effect size for completion time which is illustrated in the CI's (PC compared to C: 15 – 210 s), indicating a considerable difference in terms of change in completion time (Mauger et al., 2010). Furthermore, change in PV was only recorded pre and post TT within each condition. Given the lack of literature demonstrating a consistent increase in PV in line with the commonly used dosage of glycerol hyperhydration (Goulet et al., 2007), it is possible the PV peaked and descended before the post sample was measured. Also, additional PV time points after CWI could display any reduction caused by the pre-cooling method. Future studies are needed to pinpoint the peak change in PV and how long it takes for this to occur after ingesting a glycerol and water solution. Although the method of CWI proved to have high thermoregulatory and ergogenic benefit, it lacks practical application, future research needs to explore alternative methods which provide both

a reduction in thermoregulatory strain in the heat and is easily applicable in competition/occupational settings.

Conclusion

In summary, pre-cooling in isolation (PC) is an effective method for reducing thermal strain and improving 10 (16.1km) mile time trial performance by 6% compared to C in hot and humid environments. The combination of PC+HH however, does not provide any further ergogenic benefit compared to PC alone. Despite the improvement in thermoregulation and completion time, HSP72 mRNA expression was not significantly attenuated after pre-cooling compared to other conditions, potentially due to the exercise mode employed. Overall, the results of the present study suggest that the use of CWI as a pre-exercise intervention strategy can benefit endurance performance and perhaps occupational pursuits in the heat and humidity, although the ecological validity of such external pre-cooling methods require further elucidation, as do PV changes during and post glycerol loading.

CHAPTER 5: Experiment 2 - Optimal glycerol dosage hyperhydration effect on plasma volume.

5.1 Introduction

In the first experimental chapter glycerol hyperhydration was utilised as a hydration intervention strategy to delay the onset of dehydration and improve cycling performance in the heat and humidity. It was hypothesised that the combination of the pre-cooling strategy and glycerol hyperhydration would provide optimal performance results compared to either strategy alone. The results conflicted this however, demonstrating that glycerol hyperhydration had no ergogenic or thermoregulatory benefit; with time trial completion times (2027 ± 89 sec) and thermoregulatory parameters (see figure 4.4 and 4.5) being similar to the control condition (2063 ± 121 sec). As discussed previously in section 4.4, a possible explanation for this was that CWI significantly altered the glycerol's ability to induce osmotic gradients and retain additional water (Arnall et al., 1993). However, further examination of the literature reveals that the accepted presumption that glycerol's ergogenic effect worked through PV expansion in turn increasing sweat rate and reducing T_{Rectal} is not a robust effect.

Only two of the studies described and analysed in the review of literature table 1.1 have observed fluid retention potential of glycerol hyperhydration through PV expansion (Coutts et al., 2002; Hitchins et al., 1999; Murray et al., 1991). As mentioned previously, large heterogeneity in experimental design, ingestion times and glycerol dosages between studies makes it extremely difficult to determine optimal dosage and ingestion time.

Goulet et al. (2007) proposed after conducting a meta-analysis that the optimal dosage of glycerol to water ratio for maximising fluid retention is 1.2 g·kg BM to 21 ml·kg BM. Previous research has shown increases in plasma glycerol concentrations after Goulet's optimal dosage G-HH (Goulet, 2010) but no research has observed its effect on typical hydration parameters measured such as plasma volume at rest.

Aim

Therefore, it was the purpose of this experiment to compare hyperhydration with water and optimal glycerol dosage on peak percentage change in plasma volume and to observe the time course of any plasma volume expansion. It was hypothesised that glycerol hyperhydration would result in a significantly higher peak % change in plasma volume and maintain this expansion for a longer duration compared to water hyperhydration.

5.2 Methods

Subjects

16 recreationally active males (age: 19 ± 3 yrs, Weight: 75 ± 10 kg, urine osmolality: 423 ± 312 mOsm·Kg^{-1}) participated in this study.

Experimental Design

This was a repeated measures study design. Each participant visited the laboratory on two occasions. Each condition was completed in a temperate lab (26°C and 40% RH). See section 1.2 for hydration status necessities before participants commenced the trial.

Participants reported to the laboratory at 10:00 am, weight measurements, urine and capillary blood samples were collected. The capillary blood samples obtained from the fingertip were used to measure haematocrit and haemoglobin levels within the blood through methods stated in 3.8.1. Participants achieved hyperhydration by ingesting either 1.2 g·$kg^{-1}$of body mass (BM) glycerol mixed in 26 ml·kg^{-1} of BM of water or an equal volume water solution with aspartame flavouring on each visit. Solutions were administered evenly every 15 minutes for a 90 min period. During the ingestion period capillary blood samples were taken every 30 minutes. Following the 90 min hyperhydration, participants were seated for a further 135 min during which capillary blood samples were taken every 15 minutes. (see figure 5.1 for experimental schematic).

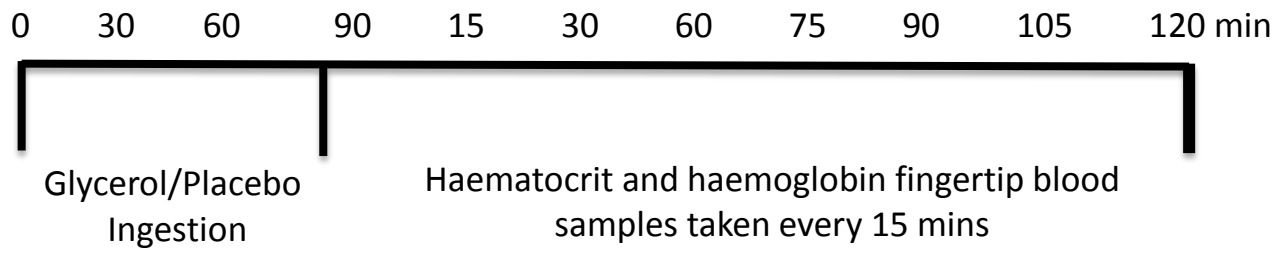


Figure 5. 1 : Experimental chapter 2 schematic.

Statistical Analysis

All data is presented as mean ± SD. Pre exercise hydration status, peak PV and %ΔPV were checked for statistical assumption using conventional graphic methods and were deemed plausible in all cases. Linear mixed models were chosen to determine if there was any difference in %ΔPV between conditions. Ninety five percent confidence intervals (95% CI) and effect sizes (ES) were also presented. Two tailed significance was accepted as $p < 0.05$.

5.3 Results

There was no significant difference in pre-trial hydration status between conditions (Table 5.1). On average participants ingested 3658 ml ± 1057 of the water (W-HH) and glycerol (G-HH) solutions.

Table 5. 1: Presents the urine osmolality of the participants in each condition.

	Glycerol hyperhydration (G-HH)	Water hyperhydration (W-HH)
UOsm (mOsmol ·kg)	443 ± 245.5	363 ± 185

There was a significant difference in the main effect of peak %ΔPV between G-HH and W-HH ($F_{1, 9.3}$ = 14.373, p = 0.004). G-HH peak %ΔPV was (19.1 ± 6.3 % higher than baseline) on average 8.8% more than W-HH (10.2 ± 4.5% increase from baseline) (p = 0.004, 95% CI = 3.59 to 14.11 %) producing a very large ES (2.01) see figure 5.2.

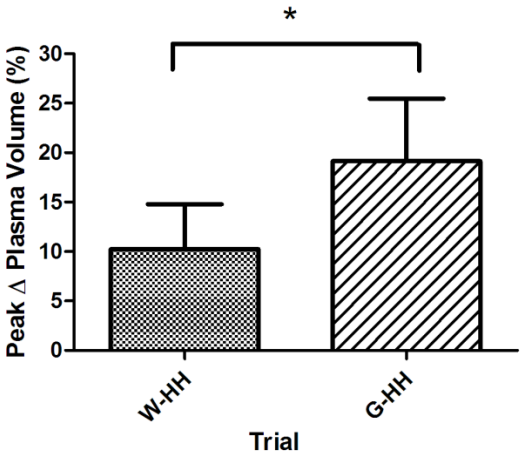


Figure 5. 2: presented are mean ± SD (*n* = 16) data for peak percentage change in plasma volume (%ΔPV) between water hyperhydration (W-HH) and glycerol hyperhydration (G-HH). * Represents significant difference between the conditions (p < 0.05)

There was significant interaction effect for %ΔPV over the observation period between hydration conditions ($F_{9,51}$ = 2.7, p = 0.01) (figure 5.3). There was overall significant difference between G-HH and W-HH throughout the 120 min period (p < 0.001, 95% CI = 3.2 to 9.6 %). From the end of the ingestion, G-HH PV was higher than that of W-HH (p < 0.001, 95% CI = 6.2 to 20.5 %) producing a very large ES (2.3), this higher PV expansion continued throughout the observation period. By the 105 min G-HH PV was 6.5% higher than W-HH (p = 0.035, 95% CI = 0.5 to 20.6 %) continuing until the completion of the trial producing a very large ES (1.69).

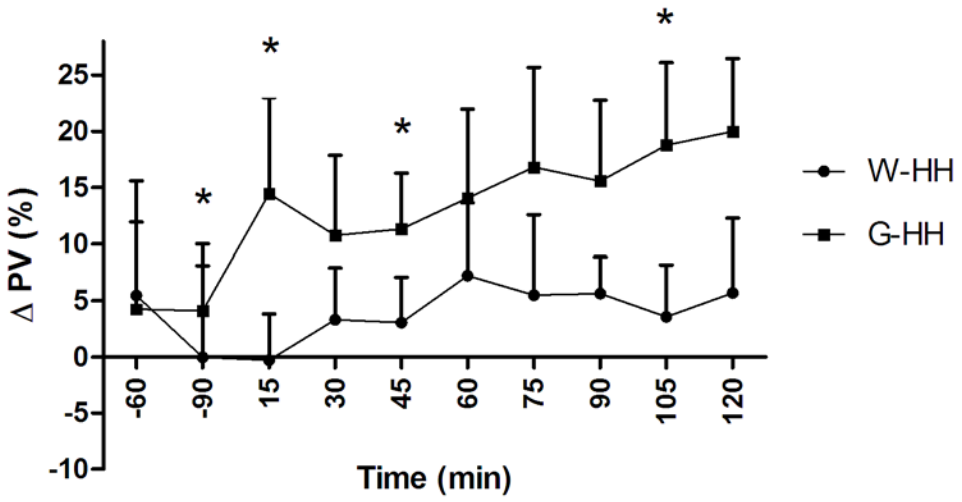


Figure 5. 3: represented are Mean ± SD (*n* = 16) for percentage change in plasma volume (%ΔPV) during ingestion (-60 and -90) and every 15 minutes for a 2 hour period post ingestion during water hyperhydration (W-HH) and glycerol hyperhydration (G-HH). *G-HH %ΔPV significantly greater than W-HH (p < 0.05).

5.4 Discussion

The purpose of this investigation was to explore an explanation for why PC + HH had no further ergogenic effect to PC alone in experimental chapter 1. This was achieved through the observation of PV expansion after glycerol hyperhydration in a sedentary state. Additionally, to establish the peak PV increase after G-HH. Primarily we found that G-HH induced a peak ΔPV of 19.1 %, averaging at 8.8% higher than W-HH in ambient environmental conditions. Secondly, peak %PV appeared 15 min post the 90 min hydration period, with raised PV levels sufficing for the full 120 min observation time frame.

There was a main effect of %ΔPV during the measured time period with two points of significance, rate of glycerol turnover (rate of appearance of glycerol) likely accounting for this response. With the solution being ingested gradually over 90 min glycerol turnover is a plausible explanation for the significant peaks of PV as is illustrated in figure 5.3. A review conducted (Riedesel et al., 1987) reported that the greatest fluid retention was on average recorded 60 – 150 min after ingestion (See figure 5.3). Supporting the results reported in the present study with significant plasma expansion observed immediately after the 90 min ingestion period and continued for the full observation time. In contrast to our study (increase of 8.8%), Hitchins et al. (1999) and Coutts et al. (2002) reported only increases in PV of 2.8% and 2.78% compared to W-HH during the 2hr hydrating period utilised by both studies. In the case of Hitchins et al. (1999) this may be as a result of the lower dosage (1 g·kg BM of glycerol and 22 ml· kg BM of water) or the short ingestion period (30 min). Glycerol turnover would reach saturation quickly, potentially leading to the renal tubes being unable to absorb as much filtered glycerol as it would if the solution was drunk over a longer period. Peak PV expansion occurred at 90 min similar to the results found in the present study.

Coutts et al. (2002) only measured post the 120 min ingestion period, our results suggest that peak PV expansion may have risen and fallen by this point.

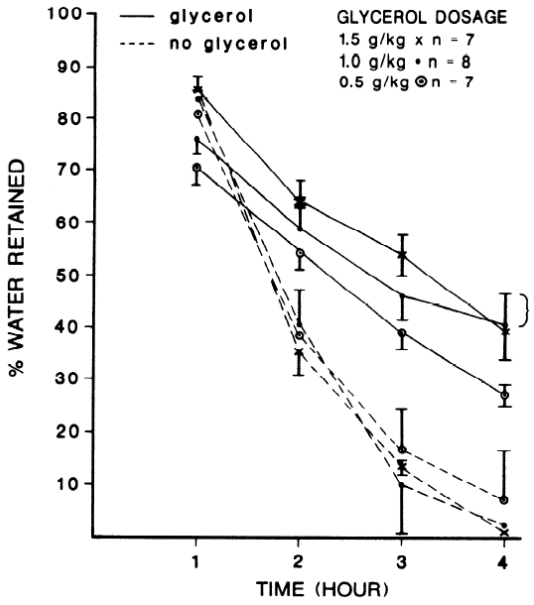


Figure 5. 4: Illustrates the % water retained after fluid solutions of varying dosages with the addition of glycerol and without (Riedesel et al., 1987).

The results from the present study support the hydration period utilised in experimental chapter 1, PV expansion began immediately after the 90 min drinking and continued to rise for the subsequent 120 min. This implies that glycerol hyperhydration should have had an ergogenic effect and theoretically should have enhanced performance when combined with the thermoregulatory benefit of pre-cooling. It is therefore likely that the dieresis effect of the CWI (Arnall et al., 1993) was responsible for altering the PV expansion and counteracting the effect of glycerol-hyperhydration.

It should be noted that due to glycerol's plasma expander capabilities it is on the WADA list of prohibited substances (Wada, 2012) both during and out of competition (See section 2.1.5 for more detail), this limits the usage of G-HH to occupational/military pursuits. The use of

G-HH within this population could be highly advantageous, particularly when on exercise and the opportunity to rehydrate is limited increasing the likelihood of EHI.

Limitations

There was large SD reported in the present study, this could represent great individual variation in fluid retention; with individual peak PV expansion after G-HH; ranging from 7 – 28%. This individual variation in hyperhydration is suggested to be due to varying glycerol space (Nelson et al., 2007), making it difficult to generalise G-HH effect for the global population. Despite the sample size (n = 16) being larger than previous research into G-HH, due to the large SD it would be advantageous to test a greater amount of participants to produce results which can be applied to the wider population. Additionally, plasma glycerol concentration was not measured, this parameter would substantiate that an increase in PV is directly as a result of the glycerol ingested. Future research should observe the combination of G-HH and CWI monitoring closely the change in PV to confirm the altering effect of the CWI to water retention and an explanation for the rejected hypothesis is experimental chapter 1. Additionally to further research needs to be conducted into the ergogenic benefit of G-HH in a military field setting.

Conclusion

These results support the use of glycerol as an effective plasma volume expander to aid hydration in occupational pursuits but its individual variation must be taken into account. Despite the significant results found in this study it is difficult to establish a specific timeframe for peak hyperhydration and PV as several physiological mechanisms effect fluid intake and retention during exercise. These results indicate that glycerol hyperhydration can increase plasma volume which in turn produces a hyperhydrated state. Implying that the ineffective response of G-HH alone and in combination with pre-cooling in experimental

chapter 1 may be as a result of the impact of CWI altering the water retention and expansion of plasma volume produced by the G-HH.

6 CHAPTER 6: Experiment 3 – Pre-cooling strategies: acetaminophen in comparison to external and internal methods in extreme heat.

6.1 Introduction

Experimental chapter 1 established that glycerol hyperhydration does not add thermoregulatory or ergogenic benefit to pre-cooling prior to exercising in hot environments. This coupled with its appearance on WADA's prohibited substances list (Wada, 2012) leads this research to focus on investigating effective pre-cooling methods. Particularly those which incorporate both high ecological validity and ergogenic benefit, which can be utilised to improve performance in an exercise and occupational setting.

Cold water immersion has proven to be the most effective method of pre-cooling for improving endurance performance and is highly advantageous for exercise in the heat >35°C (Marino, 2002; Wegmann et al., 2012). This was further supported by the results found in experimental chapter 1. Internal pre-cooling methods such as ice slurry ingestion provides an alternative method with practical application and the additional advantage of maintaining fluid balance and hydration status (Ihsan et al., 2010; Siegel et al., 2010; Stanley et al., 2010). A pharmacological agent with hypothermic potential would offer a method of pre-cooling with ergogenic effect and ease of application increasing ecological validity. Recent research by Mauger, Taylor, Harding, Foster, et al. (2013) reported a acetaminophen induced ~0.15°C reduction in T_{core} during exercise in the heat, which lead to an improved TTE of ~4mins. However, as T_{core} was not recorded prior to exercise, it is difficult to define if acetaminophen acted as a pre-cooling mechanism, or attenuated the rate of rise during the exercise.

When environmental temperatures exceed 35°C, heat loss reverses to heat gain increasing T_{core} and heat stress within the body (Brotherhood, 2008). The majority of intervention strategies researched have been conducted in <30°C ambient environmental temperature (Wegmann et al., 2012). Considering the effect of conditions exceeding 33°C on thermoregulatory system and amplified heat stress, it is important to investigate whether strategies such as pre-cooling are as effective in higher temperatures commonly experienced by the military, athletes and other occupational pursuits.

Extreme environmental heat increases the stress experienced not only through physiological responses but also on a cellular level (Connolly et al., 2004). This in combination with exercise causes substantial strain and damage to cells which can be observed through cellular biomarkers of heat-exercise stress such as HSPs, particularly HSP72. HSP72 can be used as a gauge of the stress response and provide insight into whether the pre-cooling methods commonly employed influence cellular stress.

Aim

The purpose of this study was to investigate and compare the thermoregulatory and ergogenic effect of external and internal pre-cooling methods in extreme heat (40°C). Particularly observing the hypothermic potential of acetaminophen and whether an acute dose of acetaminophen would reduce basal or attenuate the rise in $T_{core.}$ Furthermore, to investigate the pre-cooling methods effect on a cellular level via change in HSP72 mRNA expression. It was hypothesised that acetaminophen would attenuate T_{core} reducing heat strain and prove to be an effective pre-cooling method in comparison to already established methods. Secondly, HSP72 mRNA expression would be attenuated greatest from pre to post exercise compared to control, illustrating the reduced stress implemented by the intervention strategy.

6.2 Methods

Subjects

Eight recreationally active, healthy males unacclimatised to the heat participated in this study. Physical characteristics (mean ± SD) were: age 21 ± 1.5 yrs old, weight 74.6 ± 10.1 kg, height 176 ± 8.01 cm).

Preliminary Measurements

For this experiment, all participants underwent lactate threshold testing on the Woodway treadmill (Woodway, PPSS5, Med-I, Cranlea) using an incremental protocol. It consisted of commencing at 7 kmph followed by increases of 1 kmph every 3 minutes until a significant increase in blood lactate was observed for all participants. Respiratory measures were recorded by the online gas analyser (Cortex, Metalyser 3B, Cranlea), capillary samples were collected in microvettes every 2 minutes to analyse blood lactate using a blood lactate analyser (Analox, LMS).

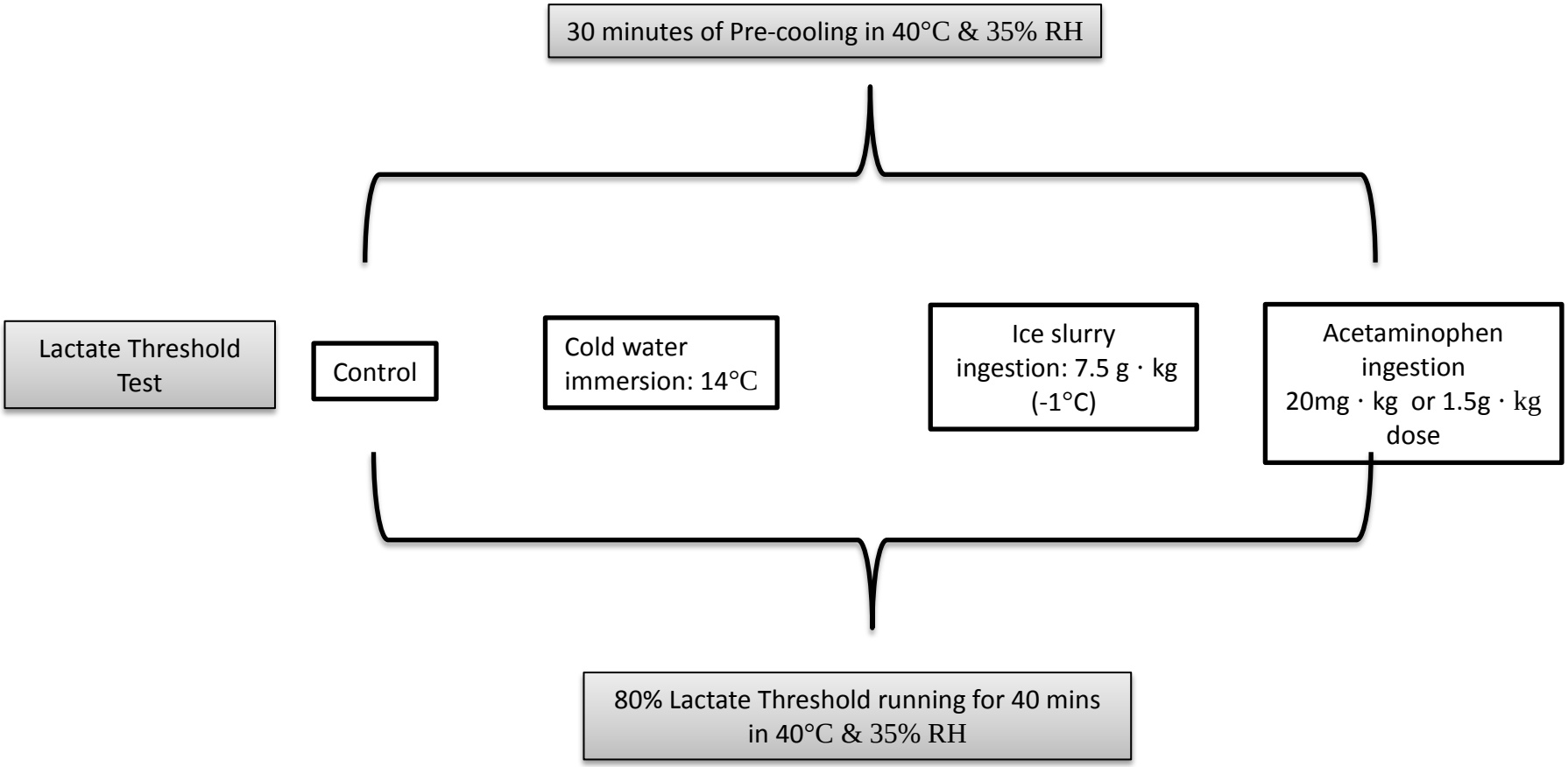


Figure 6. 1: Experimental chapter 3 overview schematic

Experimental Design

The four remaining visits to the laboratory consisted of exercise trials in extreme heat (40°C & 30% RH) after cold water immersion (CWI), ice slurry ingestion (ICE), acetaminophen ingestion (ACT) or no pre-cooling intervention (CON) (See figure 6.1). All trials were completed in an environmentally controlled chamber and separated by at least 7 days. The protocol employed was designed as a fixed intensity for a set duration adapted from the protocol used by Hillman, et al. (2011). Participants worked at 80% of their lactate threshold for 40 minutes on the Woodway treadmill (Woodway, PPSS5, Med-I, Cranlea). LT has been shown to be a more accurate predictor of endurance exercise performance then maximal oxygen uptake (VO_{2max}) (Yoshida et al., 1987). Participants were not allowed to deviate from the set intensity; exercise was ceased when participants reached ethical maximum rectal temperature (39.5°C or 2°C above basal T_{rectal}) or volitional exhaustion. The majority of participants (5 out of 8) were unable to complete the 40 minute protocol in all conditions, this made comparing and averaging data difficult. Allowing participants to deviate from the intensity when finding the conditions extremely taxing may have been a better method and prevent this limitation.

See section 1.2 for hydration status necessities of participants prior to exercise. Upon arrival to the laboratory subjects rested in a supine position for 10 minutes prior to blood sample collection (described in section 3.5). Please see general methodology, section 3.3 for information regarding attachment of temperature and heart rate equipment. Participants then completed one of the following pre-cooling methods, all of which were conducted in an environmental chamber at 40°C & 35% RH.

Cold water immersion: Participants entered the inflatable ice bath (inflatable ice bath, Gold White Fitness, Ramrugby.co.uk) situated within the environmental chamber with ambient

temperature at 40 °C and 35% RH. They were submerged up to their waists with the water averaging a temperature of 17 ± 2.3 °C for 30 minutes (Castle et al., 2006). Temperature differed from experiment 1 due to seasonal changes.

Ice slurry ingestion: Participants ingested 7.5 g/kg of body mass of ice slurry over a 30 minute period (Siegel et al., 2010). The ice slurry was divided equally into 3 portions to be ingested every 10 minutes to ensure it was ingested evenly. Participants were situated within the environmental chamber with ambient temperature at 40 °C and 35% RH throughout the 30 minute ingestion period.

Acetaminophen ingestion: Participants orally ingested 20mg/kg of BM or 1.5g (three 500 mg capsules) of acetaminophen (whichever is lower) after 15 minutes the participants entered the heat chamber where they sat in a sedentary state for 30 minutes in ambient temperatures averaging 40 °C and 35% RH. This time period of chosen as past research has indicated that peak acetaminophen concentrations occur after 45 – 60 minutes (Di Marzo et al., 2004). The ingestion occurring 15 minutes before entering the environmental chamber was employed to kept continuity between the four conditions.

T_{rectal} and Ts_{kin} were monitored continuously throughout pre-cooling. After the pre-cooling participants were escorted out of the chamber so a second venous blood sample could be taken before commencement of the 40 min fixed intensity (80% LT) run in 40°C environment. Participants were allowed 200 ml of water at 20 min point during exercise. Exercise was terminated if T_{rectal} reached ethical maximum (39.5°C) or 2°C above basal T_{rectal} or volitional exhaustion. See figure 6.2 for timing of parameter measurements such as T_{rectal}, T_{skin}, RPE, TSI, HR. Following completion of the exercise participants were removed from the environmental chamber and post exercise venous blood sample was obtained as

previously described. See section 3.6 for method of calculating HSP72 mRNA expression from venous blood samples.

Statistics Analysis

All data are presented as mean ± SD. Statistical analysis was completed using linear mixed models for repeated measures (SPSS 19.1, Chicago, IL, USA) to analyse time to peak T_{rectal}, body mass (BM) and hydration status. Condition * distance was used to examine changes in T_{rectal}, T_{skin}, HR, RPE and TSI, B[La], B[Glu], PV and HSP72 mRNA between all conditions. Ninety five percent confidence intervals (95% CI) and effect sizes were also presented. Two tailed significance was accepted as $p < 0.05$.

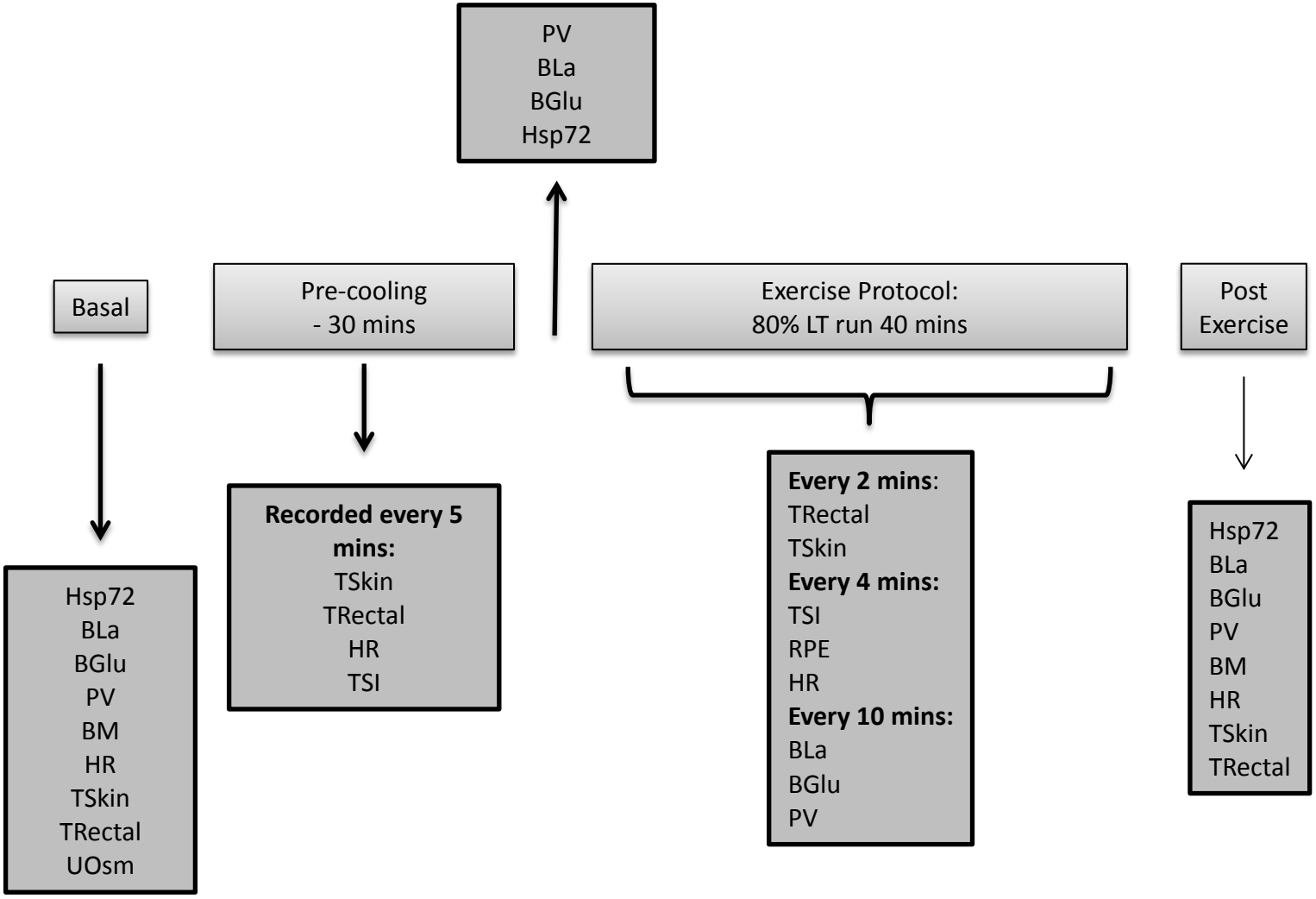


Figure 6. 2 : Experimental chapter 3 protocol schematic

6.3 Results

Thermoregulatory Response

There was significant interaction effect for T_{Rectal} over the 30 min pre-cooling period ($F_{21, 185} = 2.11$, $P = 0.05$; figure 6.3A). T_{Rectal} was higher during CWI throughout the pre-cooling period than CON, ACT and ICE. By the end of the pre-cooling period CWI T_{rectal} was on average 0.4°C lower than CON ($P = 0.003$, 95% CI = 0.136 to 0.96) displaying a small ES (0.57). There was significant interaction effect for T_{Rectal} between conditions ($F_{3, 41.5} = 6.17$, $p < 0.001$; figure 6.3B) during the exercise protocol. CWI T_{Rectal} was significantly lower on average than CON ($p = 0.03$, 95% CI = 0.03 to 1.04), ICE ($p = 0.02$, 95% CI = 0.05 to 0.97) and ACT ($p = 0.002$, 95% CI = 0.23 to 1.3). Additionally, there was significant interaction effect for peak T_{Rectal} with CWI (38.2 ± 0.4°C) peak T_{Rectal} being significantly lower than ACT (39.15 ± 0.4°C; $p = 0.04$, 95 % CI = 0.03 to 1.32; See appendix a) producing a moderate ES (1.19).

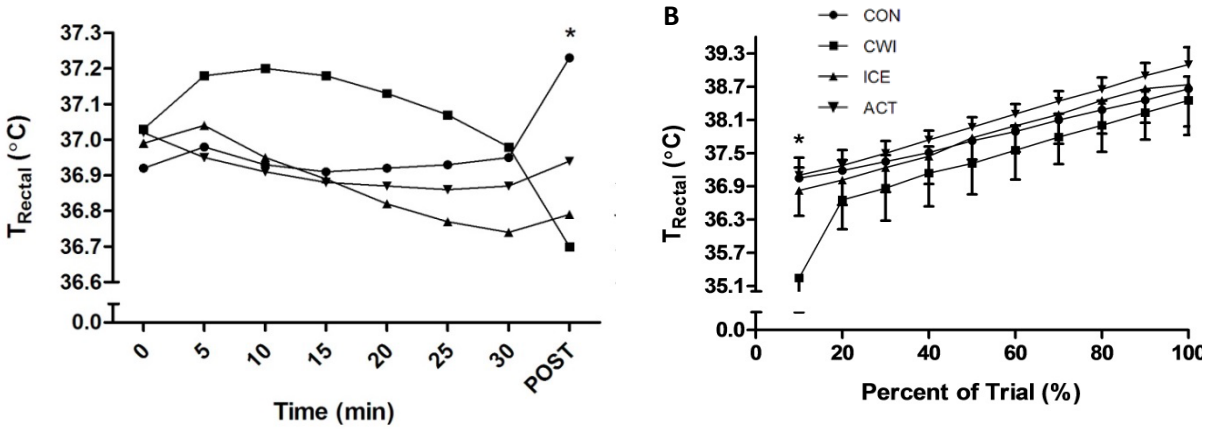


Figure 6. 3: **A**: presented means (n = 8) ± SD for T_{rectal} during pre-cooling intervention period. *Significantly lower Trectal post pre-cooling after CWI vs. CON ($p < 0.05$). Error bars omitted for clarity.
B: presented are means (n = 8) ± SD data for T_{Rectal} during exercise trial. * Significantly lower T_{Rectal} after CWI vs. CON for the first 10% of trial ($p < 0.05$).

There was significant interaction effect for T_{Skin} over the pre-cooling period ($F_{3, 29} = 50.9$, $p < 0.001$; Figure 6.4A). From the commencement of the pre-cooling period T_{skin} was lower on average 4°C in CWI compared to CON ($p < 0.001$; 95% CI = 2.54 to 5.95) producing large ES (1.72), 4.3 °C compared to ICE ($p < 0.001$, 95% CI = 1.80 to 5.71) producing a very large ES (2.08) and 3.6°C lower compared to ACT ($p < 0.001$, 95% CI = 1.8 to 5.71) producing a moderate ES (1.01) remaining lower throughout the 30 min. From the 20 min T_{Skin} was significantly lower during ACT compared to ICE by an average 1.2°C until the end of the pre-cooling period ($p = 0.03$, 95% CI = 0.43 to 2.16) producing a moderate ES (0.61). There was significant interaction effect for T_{Skin} between conditions over the exercise trial ($F_{27, 152.3} = 3.59$, $p < 0.001$; figure 6.4B). CWI T_{skin} was significantly lower compared to CON ($p < 0.001$, 95% CI = 0.48 to 1.4), ICE ($p < 0.001$, 95% CI = 0.98 to 1.82) and ACT ($p < 0.001$, 95% CI = 0.81 to 1.89) until 40% completion of the exercise trial. Peak T_{skin} was significantly lower in CWI (37.04 ± 0.6°C) compared to ICE (37.86 ± 0.7°C; $p = 0.02$, 95% CI = 0.14 to 1.94) and ACT (37.93 ± 0.5°C; $p = 0.01$, 95% CI = 0.28 to 1.96; See appendix a) both producing moderate ES (0.63 and 0.81).

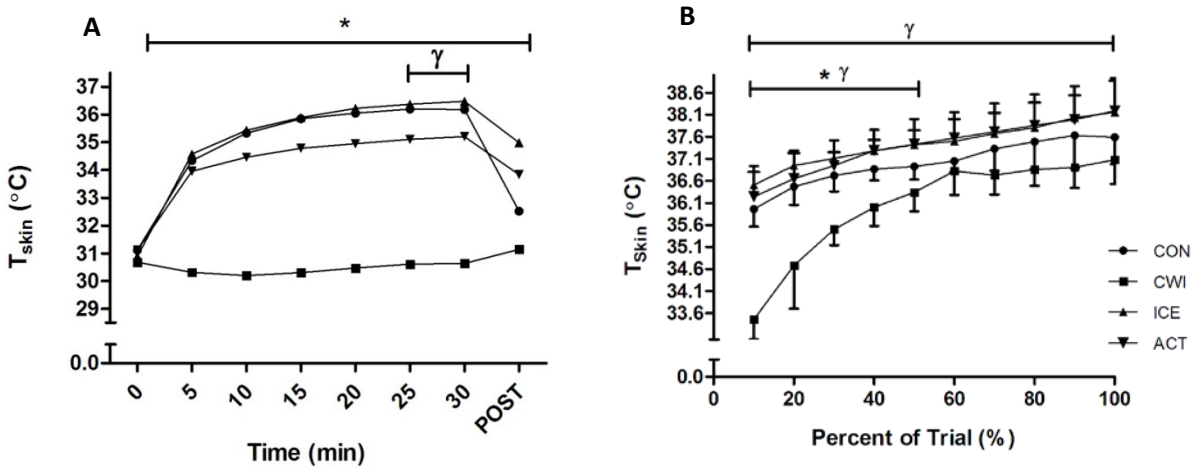


Figure 6. 4. **A**: presented means (n = 8) ± SD for T_{Skin} during pre-cooling intervention period. *Significantly lower T_{skin} throughout pre-cooling after CWI vs. CON and ICE ($p < 0.05$). ɣ Significantly lower T_{Skin} during pre-cooling after Act vs. ICE ($p < 0.05$). Error bars omitted for clarity.
B: Presented are means (n = 8) ± SD data of T_{Skin} during exercise trial. $^{\gamma}$ Significantly lower T_{skin} throughout the exercise after CWI compared to ICE and ACT ($p < 0.05$). * Significantly lower T_{skin} from the start to 60% of the trial after CWI compared to CON ($p < 0.05$).

Cardiovascular

There was significant main effect for HR during the 30 min pre-cooling period between conditions ($F_{3,12}$ = 6.06, p = 0.009; figure 6.5A). CWI HR was significantly lower than CON (p = 0.02, 95% CI = 1.55 to 20.7) and ICE (p = 0.038, 95% CI = 0.47 to 20.14) during the precooling period. There was no significant effect for HR between conditions during the exercise protocol ($F_{3,\ 44.5}$ = 9.85, p = 0.06; figure 6.5B). There was also no significant difference in peak HR between conditions ($F_{3,\ 8.81}$ = 0.71, p = 0.57; See appendix a.

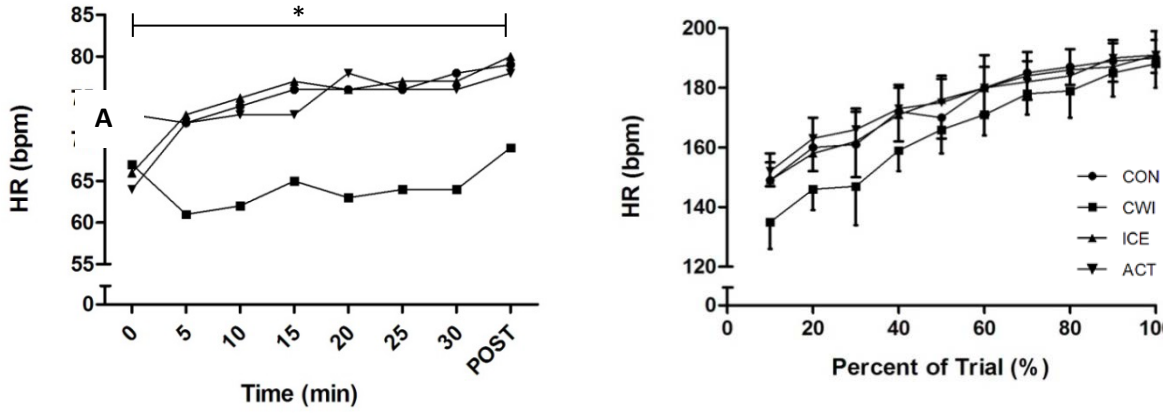


Figure 6. 5. **A**: Presented means (n =8) ±SD of heart rate (HR) during pre-cooling period. * Signficantly lower HR after CWI vs CON, ICE and ACT during pre-cooling period (p < 0.05). Error bars omitted for clarity.
B: Presented are means (n = 8) ± SD data of heart rate (HR) during exercise trial.

Thermal Perception Response

There was significant main effect for TSI between conditions over the pre-cooling period (F 21, 23.8 = 3.15, p = 0.004; see figure 6.6A). From 10 min into the pre-cooling CWI TSI was lower than CON (p < 001, 95% CI = 1.14 to 3.08), ICE (p = 0.003, 95% CI = 0.47 to 2.3) and ACT (p < 0.001, 95% CI = 0.84 to 2.94) all producing large ES (1.43 – 1.54) continuing until the end of the pre-cooling. From 15 min into the pre-cooling TSI was significantly lower for ACT compared to ICE (p = 0.049, 95% CI = 0.003 to 1.59). There was significant main effect for TSI between conditions ($F_{3,\ 44.4}$ = 14.9, p < 0.001) during the exercise trial; CWI TSI was significantly lower than CON (p < 0.001, 95% CI = 0.38 to 0.98), ICE (p < 0.001, 95% CI = 0.12 to 0.68) and ACT (p = 0.003, 95% CI = 0.13 to 0.83) (see figure 6.6B).

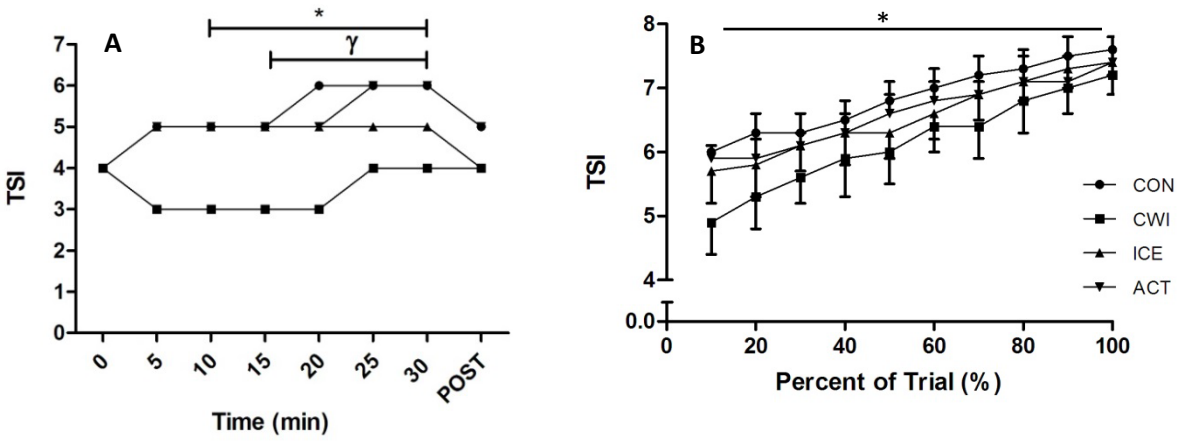


Figure 6. 6 **A**: presented means (n = 8) ± SD of TSI during pre-cooling period. * signficantly lower TSI for CWI vs. ICE and CON from 10 min to 30 min. ᵞ Signficantly lower TSI after ACT vs ICE from15 min to 30 min. Error bars omitted for clarity.
B: Presents mean (n = 8) ± SD of Thermal sensation index (TSI) during the exercise trial for all 4 conditions. * Significantly lower TSI for CWI vs. ICE, ACT and CON.

Perceived Exertion Response

There was no significant interaction effect for RPE between conditions (F 27, 148.3 = 0.33, p = 0.99 see figure 6.7) or for peak RPE (F 3, 10.21 = 1.28, p = 0.33; See appendix a).

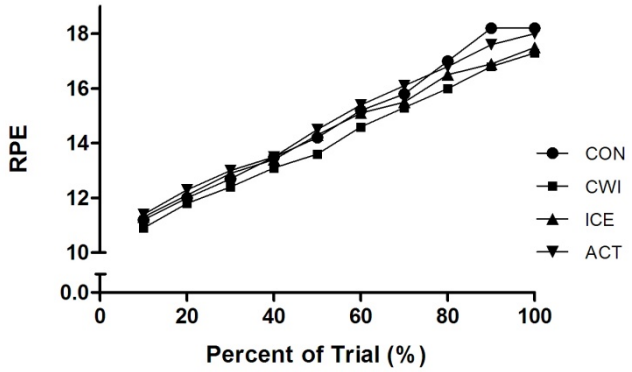


Figure 6. 7: Presents mean (n = 8) ± SD of rating of perceived exertion (RPE) during the exercise trial for all 4 conditions. Error bars omitted for clarity.

Time to peak T_{Rectal}

There was a significant interaction effect for time to peak T_{Rectal} (F 3, 10.1 = 4.11, p = 0.038; see figure 6.8) between conditions. CWI time to peak T_{Rectal} (2250 ± 254.5 sec) was significantly longer than CON (1920 ± 525.8 sec; p = 0.044, 95% CI = 0.16 to 13.19) producing a small ES (0.42).

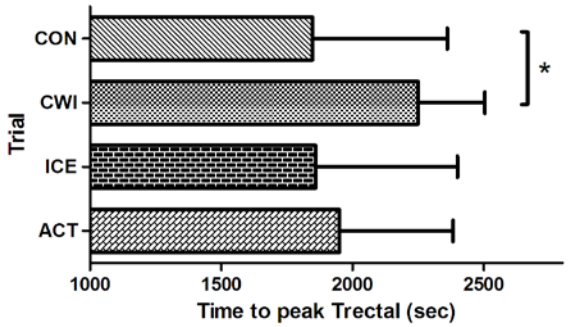


Figure 6. 8: Presented are means (n = 8) ± SD data of time to peak T_{Rectal} (°C). * Significantly longer time to peak T_{rectal} after CWI vs. CON ($p < 0.05$).

Table 6.1: Represents the urine, haemodynamic and fluid changes for all trials.

	Control		**Cold water immersion**		**Ice slurry ingestion**		**Acetaminophen ingestion**	
	Pre	post	pre	post	Pre	post	pre	post
UOsm	307.14 ± 244.52	--	404.5 ± 234.71	--	298.571 ± 145.07	--	255 ± 183.84	--
BML	--	0.47 ± 0.36	--	0.375 ± 0.23	--	0.44 ± 0.28	--	0.33 ± 0.21
%ΔPV	1.16 ± 2.51	0.01 ± 2.07	0.07 ± 3.17	1.77 ± 4.12	1.30 ± 2.69	0.57 ± 2.38	0.93 ± 4.77	3.06 ± 4.65

There was no significant effect for UOsm (urine osmolality) prior to the exercise protocol between conditions ($F_{3,21} = 1.24$ $p > 0.05$). There was no significant effect for body mass (BML) ($F_{3,21} = 0.52$, $p > 0.05$) and %ΔPV (percent change in plasma volume) ($F_{3,20.1} = 6.06$, $p = 0.32$) from pre to post exercise trial between conditions.

Hydration

There was no significant main effect for UOsm (urine osmolality) prior to the exercise protocol between conditions ($F_{3,21} = 1.24$, $p > 0.05$). There was no significant main effect for body mass loss (BML) ($F_{3,21} = 0.521$, $p > 0.05$) and %ΔPV (percent change in plasma volume) ($F_{3,\ 20.1} = 6.06$, $p = 0.32$) from pre to post exercise trial between conditions (See Table 6.1).

HSP72 Leukocyte Expression

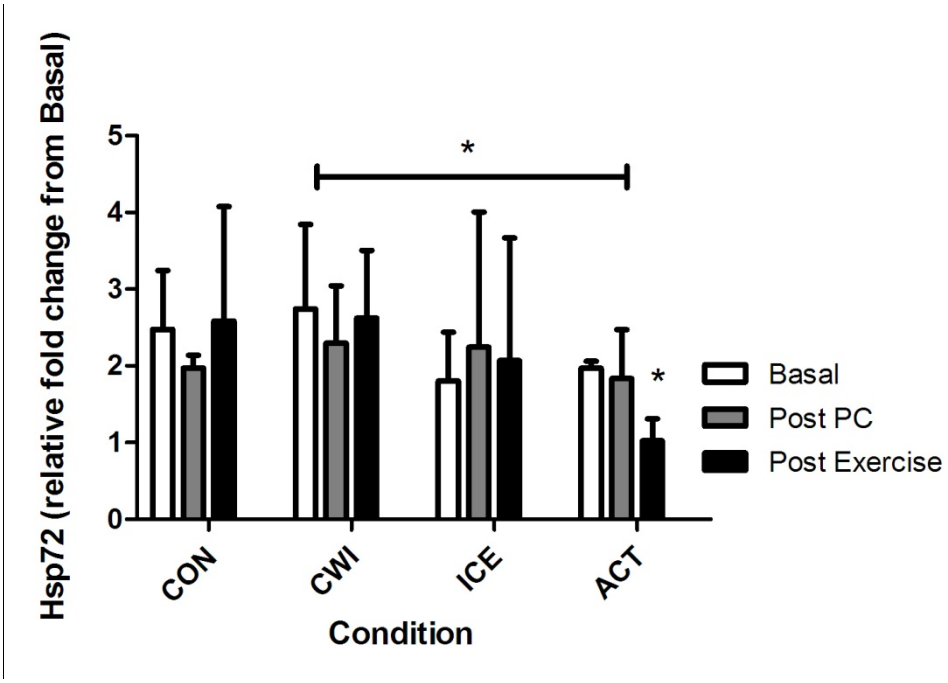


Figure 6. 9: presented are means (n = 8) ± SD data of ΔΔ CT HSP72 mRNA expression.
*significantly lower Hsp72 mRNA expression ACT compared to CWI post exercise ($p < 0.05$).

There was significant condition ($F3,18 = 5.86$, $p = 0.005$) and interaction effect ($F6,12.9 = 3.05$, $p = 0.044$) for leukocyte HSP72 mRNA expression (figure 6.9). There was a significant down regulation of HSP72 immediately post exercise after ACT compared to CWI ($p = 0.003$, 95% CI = .251 to 1.63) producing a large ES (1.38).

6.4 Discussion

The purpose of this investigation was to compare cold water immersion, ice slurry ingestion and acetaminophen pre-cooling methods and their thermoregulatory and ergogenic effect in extreme environments (40°C & 35% RH). Focusing on the effect of acetaminophen during pre-cooling period and the exercise protocol. Finally, to investigate the cellular stress response to the pre-cooling methods via HSP 72 expression. Firstly, CWI proved to be the most effective pre-cooling method investigated, with significant reduction in T_{Rectal}, T_{skin}, HR and time to peak T_{Rectal}. These findings support previous literature substantiating CWI as a highly effective pre-cooling method even in elevated heat (40°C & 30% RH) (see figures 6.3 and 6.4). It was found that an acute dose of acetaminophen did not have a significant effect on T_{Rectal}, T_{skin}, HR or TSI during the exercise trial in extreme environmental conditions, a significant reduction of T_{skin} however was observed in the pre-cooling period. Interestingly, a significant down regulation of HSP72 mRNA expression was observed post exercise in ACT condition compared to CWI.

The present study was principally designed to investigate physiological responses to exercise in the heat after pre-cooling methods with no scope for the effect on performance. However, due to the majority of participants being unable to complete the set exercise time of 40 min (5/8), we were able to have a minor insight into ergogenic benefit of the three pre-cooling mechanisms. This was presented through time to peak T_{Rectal}.

External method - Cold water immersion

The findings of this study support CWI as a highly effective pre-exercise intervention strategy to reduce thermoregulatory strain and perceived thermal stress compared to ICE and ACT (See figures 6.3, 6.4 and 6.7). Experimental chapter 1 confirmed that CWI has ergogenic benefit when exercising in 30°C, however, limited research had looked at pre-

cooling methods effect in elevated environmental conditions >35°C (Lee et al., 2008; Mitchell et al., 2003) with the addition of completing the pre-cooling within the hot environment to improve ecological validity. It is generally accepted that the primary mechanism of an external pre-cooling method is to reduce thermal heat stress via reduction in core temperature and delay the onset of fatigue/exhaustion (Wegmann et al., 2012). Although there was no reduction in T_{rectal} within the pre-cooling period during CWI, this was to be expected as a result of the afterdrop effect as previously explained in experimental chapter 1. CWI T_{Rectal} maintained at 37.18 ± 0.36 °C for the first 20 min, by the end of the pre-cooling period T_{Rectal} had reduced to 36.70 ± 0.57°C. This eventual reduction in T_{Rectal} increased the margin between starting T_{Rectal} and the critically high T_{Rectal} associated with exercise-heat induced fatigue compared to control. The combined maintenance of T_{skin} (figure 6.4), reduction in HR (figure 6.5) and T_{rectal} (figures 6.3) during the pre-cooling period increased the core-skin thermal gradient greatly, adding to the mechanism of attenuating the rise in T_{Rectal} during the exercise (figure 6.3). Throughout the exercise trial T_{Rectal} was lower after CWI than ACT, ICE and CON further confirming CWI as an effective method to reduce thermal strain. Figure 6.8 illustrates that time to peak T_{Rectal} was longest after CWI (2250 ± 254 sec) with the lowest peak T_{Rectal} (38.45 ± 0.47°C) as would be expected with its ability to reduce thermal heat stress and delay the onset of fatigue. Despite the effective benefit of CWI on physiological mechanisms, from a cellular perspective there was no attenuation in HSP72 expression post exercise compared to CON. This means there is high thermal strain within the cell regardless of the reduced thermal stress physiologically.

Ice slurry ingestion

Ice slurry ingestion reduced T_{rectal} by 0.4°C compared to CON ($p = 0.003$, 95% CI = 0.136 to 0.96) during the pre-cooling period. This did not however, continue once exercise commenced. Ice slurry ingestion did not prove to be an effective pre-cooling method for

exercising in the heat within our study, conflicting previous findings (Ihsan et al., 2010; Siegel et al., 2010). Ihsan et al. (2010) observed a 6.5% improvement in 40km TT performance after ice slurry ingestion and a 1.1 ± 0.59°C reduction in T_{core} after the pre-cooling period compared with control. Similar to our study, no significant differences existed in T_{skin}, RPE and HR compared to control. Additionally, the rate of change in T_{rectal} rapidly diminished and by the end of the exercise it was higher than control. The reduction in T_{core} during the pre-cooling period reported in Ihsan et al. (2010) study (1.1°C) was substantially higher than observed in this study (0.4°C), this may be due to the method of gastrointestinal measurement being utilised. The use of gastrointestinal pills to measure T_{core} after the ingestion of cold fluids, could lead to distorted measurements as a result of the direct effect of the ice slurry on the pill (O'Brien et al., 1998; Wilkinson et al., 2008). This would appear as significantly lower T_{core} compared to results gained from T_{Rectal} measurements.

Furthermore, to increase practicality within this study the pre-cooling mechanisms were performed within the environmental chamber (40°C & 30% RH). Within such an environment the thermal gradient would be altered, causing greater heat absorption and therefore attenuating the cooling mechanism of the ice slurry (Siegel et al., 2012). If the pre-cooling was performed in a cooler environment, ice slurry ingestion ability to reduce thermal heat stress may have been improved. Siegel et al. (2012) also performed pre-cooling methods in the warmer environment and found the same limitations to ice slurry's effectiveness, additionally they reported a reduction of T_{rectal} 0.43 ± 0.14°C near identical to the reductions witnessed in the present study.

Acetaminophen ingestion

Acetaminophen did not prove to be an effective pre-cooling agent within this study conflicting recent research from Mauger, Taylor, Harding, Wright, et al. (2013) which

reported small yet significant reduction in T_{Rectal} (-0.15°C) and T_{skin} (-0.47°C) and improved performance. A plausible explanation of these differing results is that in the present study participants were within a heated environment for 30 min prior to exercise to replicate the procedure used for CWI and ICE. As mentioned previously, for ICE, the addition of heat may have altered the thermal gradient reducing any potential pre-exercise hypothermic mechanism from occurring (Siegel et al., 2012). Interestingly during the pre-cooling period T_{skin} was significantly reduced by 1.2°C for the final 10 min after ACT compared to ICE. It is unusual to see a decreased T_{skin} in the absence of reduced T_{rectal} particularly after an internal form of pre-cooling. This could suggest that acetaminophen act upon the vasomotor mechanisms, causing vasoconstriction (Charkoudian, 2010).

A reduction of 0.3 ± 0.3°C compared to CWI was recorded however this due to the afterdrop phenomenon of the CWI rather than a hypothermic effect of the ACT. Despite this, there was still a noticeable trend of T_{rectal} reduction during the pre-cooling period in comparison to CON which may indicate a worthy hypothermic action. It should however, be taken into account that particularly after ACT the thermoregulatory parameters had large SD possibly due to high inter-individual variation associated with acetaminophen as illustrated in previous acetaminophen studies (Critchley et al., 1986). Interestingly, a significant down regulation of HSP72 mRNA expression was observed post exercise after ACT compared to CWI's post exercise expression (see figure 6.8). It is known that the difference is not due to the cold stressor effect of the CWI as CWI HSP72 expression levels were consistent with CON at all-time points. A potential explanation for this finding is that acetaminophen can prevent oxidative burst and delay apoptosis, subsequently reducing cellular stress and heat shock response (Freitas et al., 2013). To date Freitas et al. (2013) is the only study to investigate acetaminophen's effect on neutrophils in the event of an inflammatory process. It was reported that acetaminophen efficiently modulates neutrophils' oxidative burst additionally

affecting cell death leading to a delay in apoptosis. As previously described in the review of literature section 2.1.5 exercise (enhanced in hot environments) has a pyrogenic effect stimulating a release of inflammatory cytokines activating neutrophil production (Martin et al., 1997). This enhanced neutrophil production during exercise infers that acetaminophen's potential to directly modulating oxidative burst could be a plausible explanation for the reduced cellular stress witnessed in the present study.

Limitations

A limitation of experimental chapter 3 is that plasma acetaminophen concentrations were not measured after the ingestion period. This would have given an insight into inter-individual metabolism rates of acetaminophen and explain why participants had varying responses. Additionally, due to time restrictions a familiarisation of the exercise protocol was not completed, only 3/8 participants were able to complete the 40 min fixed intensity exercise on all four occasions. If a familiarisation had been conducted it would have become apparent earlier that in such extreme heat this protocol was not realistic for recreationally active participants and it could be altered prior to experimental testing. The average T_{Rectal} of all conditions was 38.73°C by the end of the exercise protocol. Participants perceived they had reached extreme heat stress (TSI) however, peak T_{Rectal} was lower than what is expect in association with exercise/heat stress induced fatigue (Nielsen et al., 1993). In future, it may be advantageous to use participants of a higher fitness level or a different exercise protocol which may result in an alter outcome. Unlike the present study, Mauger et al. (2010) exercise protocol was self-paced, with their findings it was proposed that the capacity of acetaminophen to enhance pain tolerance is a possible variable that is used by the central governor system (CGS) to reduced exercise intensity as a protective mechanism against physiological harm. Unlike our study however the focus was to observe the performance effect of acetaminophen's hypothermic potential rather than the physiological.

Conclusion

In conclusion this study demonstrates that an acute dose of acetaminophen is not an effective alternate pre-cooling method to cold water immersion when exercising at fixed intensity and duration in extreme heat (40°C). Regardless of its enhanced practical application, ice slurry ingestion did not elicit a significant effect on the thermoregulatory system during exercise. Despite the observed lack of thermoregulatory effect of acetaminophen, there was a noticeable reduction in cellular stress through the possible prevention of oxidative burst. Future work needs to determine the mechanisms by which acetaminophen works on individuals exercising in the heat and to define the variation in response to acetaminophen between individuals. Subsequently, determining a peak ingestion time and how long this suffices for by measuring serum acetaminophen concentration. This may help to establish an explanation for the differing results from the present study and Mauger, Taylor, Harding, Wright, et al. (2013).

CHAPTER 7: General discussion and conclusion

7.1 General discussion

The objectives and aims set for each experimental chapter are reiterated below with a conclusive statement of whether they have been determined.

Experimental Chapter 1 - Molecular and performance effects of pre-cooling and hydration strategies

1) To investigate the effect of combined hyperhydration and pre-cooling on 10 mile TT cycling performance in hot and humid conditions

   - Hyperhydration had no additional performance or thermoregulatory benefit to pre-cooling alone, contrary to hypothesis.

2) Secondly to investigate HSP72 mRNA expression as a thermal stress marker between conditions.

   - *There was no significant difference in HSP72 mRNA expression between conditions*

Experimental Chapter 2 - Glycerol hyperhydration effects on plasma volume

1) To investigate the peak % change in plasma volume after glycerol hyperhydration compared to water hyperhydration.

   - *Glycerol hyperhydration peak %ΔPV was significantly higher than water hyperhydration.*

2) Secondly, to observe the time course of plasma volume expansion suffices for after glycerol hyperhydration in sedentary state.

   - *Glycerol hyperhydration maintained plasma volume expansion for at least 120 min.*

Experimental Chapter 3 - Molecular and thermophysiological effects of acetaminophen as a pre-cooling agent

1) To compared the thermoregulatory effect of external and internal pre-cooling methods including acetaminophen on sub maximal exercise in extreme heat.
    - *Cold water immersion was reiterated as the most effective form of pre-cooling for reducing thermal strain and delaying the onset of fatigue compared to ice slurry ingestion and acetaminophen.*
    - *Acetaminophen had no hypothermic effect with any noticeable reduction in thermal strain during the exercise.*
2) Secondly to investigate HSP72 mRNA expression as a thermal stress marker between conditions exercise in extreme heat.

    Acetaminophen significantly down regulated HSP72 mRNA expression post exercise compared to cold water immersion, contrary to hypothesis.

Taking together the findings of these three experimental chapters, primarily they show that cold water immersion is the most thermoregulatory effective pre-cooling method both at 30°C & 50% RH and 40°C & 30% RH. It significantly reduced T_{Skin} and HR during pre-cooling and T_{Rectal} once exercise had begun in both experimental studies 1 and 3. It also had a significant ergogenic effect in study 1 reducing time to completion of the 10 mile TT. Despite cold water immersion's effectiveness its practical application has always been questioned due to the logistics of such a technique in field settings and incorporating its use around competition warm up (Uckert & Joch, 2007). This lead to the investigation in to more practical method of pre-cooling with recent research focusing on internal methods such as ice slurry ingestion (Ihsan et al., 2010; Siegel et al., 2010; Siegel et al., 2012). Experimental

study 3 found no thermoregulatory benefit of ice slurry ingestion; a significant reduction of T_{Rectal} was witnessed in the pre-cooling period but as soon as exercise commenced any thermoregulatory benefit was lost. As mentioned previously the ineffectiveness of ice slurry and also acetaminophen may have been due to the pre-cooling methods taking place in the hot environment (40°C and 30% RH) altering the thermal gradient hindering heat dissipation (Siegel et al., 2012). The investigation into acetaminophen's pre-cooling potential proved to find no thermoregulatory benefit during sub-maximal exercise in extreme heat. Conflicting the significant results reported by Mauger, Taylor, Harding, Wright, et al. (2013) of reduced T_{Rectal} and T_{skin} during exercise in the heat after acetaminophen ingestion. As previously mentioned the addition of ingesting in a heated environment may have augmented any potential hypothermic action prior to exercise. Additionally the exercise protocol employed may be a factor in the differing results. Despite the lack of physiological response there was a significant down regulation in HSP72 mRNA expression in leukocytes post exercise after acetaminophen ingestion compared to cold water immersion. This indicates that acetaminophen may effectively reduce cellular stress through the modulation of neutrophil's oxidative burst (Freitas et al., 2013).

The combination of hyperhydration and pre-cooling strategies did not prove to enhance thermoregulation and ergogenic benefit any more than pre-cooling alone. The results from experimental study 2 confirm that glycerol hyperhydration has a significant plasma expanding effect in moderate conditions, indicating that the thermal strain of exercising in the heat outweighs the plasma volume expansion induced by glycerol hyperhydration prior to exercise. The increased diuresis effect of the cold water immersion was also a potential explanation for the lack of plasma volume expansion during the exercise protocol in the heat. Experimental chapter 2 proved that glycerol hyperhydration produced a robust plasma volume expansion, this could be utilised as an effective intervention strategy within a military

setting. As previously described dehydration is a fundamental issue during training and operation, particularly in extreme environments. With the limited opportunities to hydrate during, it would be highly advantageous to hyperhydrate prior to the exercise delaying the onset of dehydration.

Experimental limitations

Please see relevant experimental chapter discussion for experimental limitations.

7.2 Future Recommendations

As a result of the findings and also the limitations of the current thesis, it is believed future research should be conducted. Clarification of the mechanisms of action of acetaminophen and the kinetics of potential hypothermic action need to be addressed, the findings of which may help to explain why no hypothermic effect was observed in this study but a cellular response was. Utilising hyperhydration isolation and acetaminophen ingestion intervention strategies within a military field setting would determine whether they are viable and practical methods within such an environment to elicit significant improvements in performance and reduce symptoms of EHI. It has not been determined within this study if HSP72 mRNA expression in leukocytes can be used as an effective molecular gauge of stress response, it is probable that HSP72 mRNA expression is T_{core} dependent. The exercise protocols utilised within experimental chapter 1 and 3 were not sufficient to increase Tcore over 38.5°C for a substantial period of time as described by Selkirk et al (2009) necessary to activate significant HSP72 expression.

7.3 Conclusion

It is clear that thermal strain and its effect on the body's thermoregulatory and cardiovascular systems has a detrimental effect on exercise performance and accelerates the onset of fatigue. The implication of cold water immersion as a pre-cooling strategy has proven to be effective in reducing thermal strain and increase exercise capacity significantly more than internal methods such as ice slurry ingestion and the novel use of acetaminophen but fundamentally lacks practical application in a field setting. The addition of glycerol hyperhydration to pre-cooling has no further ergogenic benefit due to the dieresis effect of cold water immersion augmenting the plasma volume expansion elicited by the glycerol hyperhydration. Despite this, glycerol hyperhydration does causes a robust increase in plasma volume, suggesting it could be a practical method to hyperhydrate military personnel prior to training and operation in extreme environments. acetaminophen did not prove to have physiologically hypothermic actions, conflicting results from previous research (Mauger, Taylor, Harding, Wright, et al., 2013). However, acetaminophen significantly reduced HSP72 expression post exercise regardless of not having a physiological effect it may have a cellular effect on reducing thermal strain. This is potentially due to its ability to delay oxidative burst through the modulation of neutrophils. HSP72 mRNA expression in leukocytes. Further research needs to be conducted into acetaminophen's pre-cooling effect to determine if the conflicting results were down to differing protocols or the higher environmental temperature in the present study (40°C). These findings highlight the importance of intervention strategies prior to exercise in hot environments particularly in occupational pursuits to reduce thermal stress put upon the complex integration of systems involved in thermal homeostasis as well as the damage on a molecular level.

CHAPTER 8: References

Adams, W. C., Mack, G. W., Langhans, G. W., & Nadel, E. R. (1992). Effects of varied air velocity on sweating and evaporative rates during exercise. *Journal of Applied Physiology, 73*(6), 2668-2674.

Anderson, M. J., Cotter, J. D., Garnham, A. P., Casley, D. J., & Febbraio, M. A. (2001). Effect of glycerol-induced hyperhydration on thermoregulation and metabolism during exercise in the heat. *International Journal of Sport Nutrition and Exercise Metabolism, 11*(3), 315-333.

Armstrong, M. J., & Maresh, C. M. (2003). The exertional heat illness: A risk of athletic participation. *Medical Journal of Exercise Nutrition and Health, 2*, 125-134.

Arnall, D. A., & Goforth, H. W. (1993). Failure to reduce body-water loss in cold-water immersion by glycerol ingestion. *Undersea & Hyperbaric Medicine, 20*(4), 309-320.

Ayoub, S. S., Botting, R. M., Goorha, S., Colville-Nash, P. R., Willoughby, D. A., & Ballou, L. R. (2004). Acetaminophen-induced hypothermia in mice is mediated by a prostaglandin endoperoxide synthase 1 gene-derived protein. *Proceedings of the National Academy of Sciences of the United States of America, 101*(30), 11165-11169.

Baler, R., Welch, W. J., & Voellmy, R. (1992). Heat-shock gene-regulation by nascent polypeptides and denatured proteins - hsp70 as a potential autoregulatory factor. *Journal of Cell Biology, 117*(6), 1151-1159.

Bandell, M., Macpherson, L. J., & Patapoutian, A. (2007). From chills to chilis: Mechanisms for thermosensation and chemesthesis via thermotrps. *Current Opinion in Neurobiology, 17*(4), 490-497.

Bergh, U., & Ekblom, B. (1979). Physical performance and peak aerobic power at different body temperatures. *Journal of Applied Physiology, 46*(5), 885-889.

Bishop, D. (2003) Warm up: potential mechanisms and the effects of passive warm up on exercise performance. *Journal of Sports Medicine 33(6)*: 439-454.

Blake, M. J., Gershon, D., Fargnoli, J., & Holbrook, N. J. (1990). Discordant expression of heat-shock protein messenger-rnas in tissues of heat-stressed rats. *Journal of Biological Chemistry, 265*(25), 15275-15279.

Bogerd, N., Perret, C., Bogerd, C. P., Rossi, R. M., & Daanen, H. A. M. (2010). The effect of pre-cooling intensity on cooling efficiency and exercise performance. *Journal of Sports Sciences, 28*(7), 771-779.

Booth, J., Marino, F., & Ward, J. J. (1997). Improved running performance in hot humid conditions following whole body precooling. [Article]. *Medicine and Science in Sports and Exercise, 29*(7), 943-949.

Borg, G. (1982). Psychophysical bases of percieved exertion. *Medicine and Science in Sports and Exercise, 14*(3), 377-381.

Botting, R., & Ayoub, S. S. (2005). Cox-3 and the mechanism of action of paracetamol/acetaminophen. *Prostaglandins Leukotrienes and Essential Fatty Acids, 72*(2), 85-87.

Bouchama, A., & Knochel, J. P. (2002). Heatstroke. *New England Journal of Medicine, 34*(6), 1978-1988.

Boulant, J. A. (2000). Role of the preoptic-anterior hypothalamus in thermoregulation and fever. *Clinical Infectious Diseases, 31*, S157-S161.

Boysen, G., & Christensen, H. (2001). Stroke severity determines body temperature in acute stroke. *Stroke, 32*(2), 413-417.

Bradford, C. D., Cotter, J. D., Thorburn, M. S., Walker, R. J., & Gerrard, D. F. (2007). Exercise can be pyrogenic in humans. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 292*(1), R143-R149.

Brotherhood, J. R. (2008). Heat stress and strain in exercise and sport. *Journal of Science and Medicine in Sport, 11*(1), 6-19.

Burton, A. C., & Bazett, H. C. (1936). A study of the average temperature of the tissues, of the exchanges of heat and vasomotor responses in man by means of a bath calorimeter. *American Journal of Physiology, 117*(1), 36-54.

Byrne, C., Lee, J. K. W., Chew, S. A. N., Lim, C. L., & Tan, E. Y. M. (2006). Continuous thermoregulatory responses to mass-participation distance running in heat. *Medicine and Science in Sports and Exercise, 38*(5), 803-810.

Caputa, M., Feistkorn, G., & Jessen, C. (1986). Effects of brain and trunk temperatures on exercise performance in goats. *Pflugers Archiv-European Journal of Physiology, 406*(2), 184-189.

Castillo, J., Martinez, F., Leira, R., Prieto, J. M., Lema, M., & Noya, M. (1994). Mortality and morbidity of acute cerebral infarction related to temperature and basal analytic parameters. *Cerebrovascular Diseases, 4*(2), 66-71.

Castle, P. C., Macdonald, A. L., Philp, A., Webborn, A., Watt, P. W., & Maxwell, N. S. (2006). Precooling leg muscle improves intermittent sprint exercise performance in hot, humid conditions. *Journal of Applied Physiology, 100*(4), 1377-1384.

Chang, W. H., Hu, S. P., Huang, Y. F., Yeh, T. S., & Liu, J. F. (2010). Effect of purple sweet potato leaves consumption on exercise-induced oxidative stress and il-6 and hsp72 levels. *Journal of Applied Physiology, 109*(6), 1710-1715.

Charkoudian, N. (2010). Mechanisms and modifiers of reflex induced cutaneous vasodilation and vasoconstriction in humans. *Journal of Applied Physiology, 109*(4), 1221-1228.

Cheung, S. S., McLellan, T. M., & Tenaglia, S. (2000). The thermophysiology of uncompensable heat stress - physiological manipulations and individual characteristics. *Sports Medicine, 29*(5), 329-359. *Exercise and Sport Science Reviews*, 32 (3), 100-106.

Cheung, S. S., & Robinson, A. M. (2004). The influence of upper-body pre-cooling on repeated sprint performance in moderate ambient temperatures. *Journal of Sports Sciences, 22*(7), 605-612.

Cheung, S.S & Gordan, S. (2004) Multiple triggers for hyperthermic fatigue and exhaustion.

Cheuvront, S. N., Ely, B. R., Kenefick, R. W., Michniak-Kohn, B. B., Rood, J. C., & Sawka, M. N. (2009). No effect of nutritional adenosine receptor antagonists on exercise performance in the heat. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 296*(2), R394-R401.

Chomczynski, P., & Sacchi, N. (1987). Single-step method of rna isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *ANALYTICAL BIOCHEMISTRY 162*, 156-159.

Christians, E. S., Yan, L. J., & Benjamin, I. J. (2002). Heat shock factor 1 and heat shock proteins: Critical partners in protection against acute cell injury. *Critical Care Medicine, 30*(1), S43-S50.

Cohen, J. (1992). A power primer. *Psychological Bulletin, 112*(1), 155-159.

Colin, J., Timbal, J., Houdas, Y., Boutelie.C, & Guieu, J. D. (1971). Computation of mean body temperature from rectal and skin temperatures. *Journal of Applied Physiology, 31*(3), 484-&.

Connolly, P. H., Caiozzo, V. J., Zaldivar, F., Nemet, D., Larson, J., Hung, S. P., . . . Cooper, D. M. (2004). Effects of exercise on gene expression in human peripheral blood mononuclear cells. *Journal of Applied Physiology, 97*(4), 1461-1469.

Cotter, J. D., Sleivert, G. G., Roberts, W. S., & Febbraio, M. A. (2001). Effect of pre-cooling, with and without thigh cooling, on strain and endurance exercise performance in the heat. [Article; Proceedings Paper]. *Comparative Biochemistry and Physiology a-Molecular and Integrative Physiology, 128*(4), 667-677.

Coutts, A., Reaburn, P., Mummery, K., & Holmes, M. (2002). The effect of glycerol hyperhydration on olympic distance triathlon performance in high ambient temperatures. *International Journal of Sport Nutrition and Exercise Metabolism, 12*(1), 105-119.

Crandall, C. G., & Gonzalez-Alonso, J. (2010). Cardiovascular function in the heat-stressed human. *Acta Physiologica, 199*(4), 407-423.

den Hertog, H. M., van der Worp, H. B., van Gemert, H. M. A., Algra, A., Kappelle, L. J., Van Gijn, J., . . . Investigators, P. (2009). The paracetamol (acetaminophen) in stroke (pais) trial: A multicentre, randomised, placebo-controlled, phase iii trial. *Lancet Neurology, 8*(5), 434-440.

Deuster, P. A., Smith, D. J., Smoak, B. L., Montgomery, L. C., Singh, A., & Doubt, T. J. (1989). Prolonged whole-body cold water immersion - fluid and ion shifts. *Journal of Applied Physiology, 66*(1), 34-41.

Di Marzo, V., Bifulco, M., & De Petrocellis, L. (2004). The endocannabinoid system and its therapeutic exploitation. *Nature Reviews Drug Discovery, 16*(2), 115-124.

Digel, I. (2011). Primary thermosensory events in cells. In M. S. Islam (Ed.), *Transient receptor potential channels* (Vol. 704, pp. 451-468).

Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma and red-cells in dehydration. *Journal of Applied Physiology, 37*(2), 247-248.

Dini, M., Corbianoco, S., Rossi, B., & Lucacchini, A. (2007). Hyperhydrating with glycerol: Effects on thermoregulation, hydration and athletic performance during specific exergonic exercise in a warm-humid environment. *Sport Sciences for Health, 2*, 1 - 7.

Dippel, D. W. J., van Breda, E. J., van Gemert, H. M. A., van der Worp, H. B., Meijer, R. J., Kappelle, L. J., . . . Invest, P. (2001). Effect of paracetamol (acetaminophen) on body temperature in acute ischemic stroke - a double-blind, randomized phase ii clinical trial. *Stroke, 32*(7), 1607-1612.

Drust, B., Cable, N. T., & Reilly, T. (2000). Investigation of the effects of the pre-cooling on the physiological responses to soccer-specific intermittent exercise. *European Journal of Applied Physiology and Occupational Physiology, 81*(1-2), 11-17.

Duffield, R., Dawson, B., Bishop, D., Fitzsimons, M., & Lawrence, S. (2003). Effect of wearing an ice cooling jacket on repeat sprint performance in warm/humid conditions. *British Journal of Sports Medicine, 37*(2), 164-169.

Duffield, R., Green, R., Castle, P., & Maxwell, N. (2010). Precooling can prevent the reduction of self-paced exercise intensity in the heat. [Article]. *Medicine and Science in Sports and Exercise, 42*(3), 577-584.

Edholm, O. G., Fox, R. H., & Macpherson, R. K. (1957). Vasomotor control of the cutaneous blood vessels in the human forearm. *Journal of Physiology-London, 139*(3), 455-465.

El Helou, N., Tafflet, M., Berthelot, G., Tolaini, J., Marc, A., Guillaume, M., . . . Toussaint, J. F. (2012). Impact of environmental parameters on marathon running performance. *Plos One, 7*(5).

Febbraio, M. A., & Koukoulas, I. (2000). Hsp72 gene expression progressively increases in human skeletal muscle during prolonged, exhaustive exercise. *Journal of Applied Physiology, 89*(3), 1055-1060.

Fehrenbach, E., Niess, A. M., Veith, R., Dickhuth, H. H., & Northoff, H. (2001). Changes of hsp72-expression in leukocytes are associated with adaptation to exercise under conditions of high environmental temperature. *Journal of Leukocyte Biology, 69*(5), 747-754.

Fehrenbach, E., Passek, F., Niess, A. M., Pohla, H., Weinstock, C., Dickhuth, H. H., & Northoff, H. (2000). Hsp expression in human leukocytes is modulated by endurance exercise. *Medicine and Science in Sports and Exercise, 32*(3), 592-600.

Fox, R. H., & Hilton, S. M. (1956). Sweat gland activity as a contributory factor to heat vasodilatation in the human skin. *Journal of Physiology-London, 133*(3), P68-P69.

Frank, M. S. B., Hilty, M. D., & Nahata, M. C. (1981). Glycerol- a review of its pharmacology, pharmacokinetic, adverse reactions and clinical. *Pharmacotherapy, 1*(2), 147-160.

Freund, B. J., Montain, S. J., Young, A. J., Sawka, M. N., DeLuca, J. P., Pandolf, K. B., & Valeri, C. R. (1995). Glycerol hyperhydration: Hormonal, renal, and vascular fluid responses. [Article]. *Journal of Applied Physiology, 79*(6), 2069-2077.

Galloway, S. D. R., & Maughan, R. J. (1997). Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. *Medicine and Science in Sports and Exercise, 29*(9), 1240-1249.

Gardner, J. W., Kark, J. A., Karnei, K., Sanborn, J. S., Gastaldo, E., P., B., & Wenger, C. (1996). Risk factors predicting exertional heat illness in male marine corps recruits. *Medicine and Science in Sport and Exercise, 28*(8), 939-944.

Geladas, N., & Banister, E. W. (1988). Effect of cold air inhalation on core temperature in exercising subjects under heat-stress. *Journal of Applied Physiology, 64*(6), 2381-2387.

Gonzalez-Alonso, J. (2012). Human thermoregulation and the cardiovascular system. *Experimental Physiology, 97*(3), 340-346.

Gonzalez-Alonso, J., & Calbet, J. A. L. (2003). Reductions in systemic and skeletal muscle blood flow and oxygen delivery limit maximal aerobic capacity in humans. *Circulation, 107*(6), 824-830.

Gonzalez-Alonso, J., Crandall, C. G., & Johnson, J. A. (2008). The cardiovascular challenge of exercising in the heat. *Journal of Physiology-London, 586*(1), 45-53.

Gonzalez-Alonso, J., Teller, C., Andersen, S. L., Jensen, F. B., Hyldig, T., & Nielsen, B. (1999). Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *Journal of Applied Physiology, 86*(3), 1032-1039.

Goulet, E. D. B. (2010). Glycerol-induced hyperhydration: A method for estimating the optimal load of fluid to be ingested before exercise to maximize endurance performance. *Journal of Strength and Conditioning Research, 24*(1), 74-78.

Goulet, E. D. B., Aubertin-Leheudre, M., Plante, G. E., & Dionne, I. J. (2007). A meta-analysis of the effects of glycerol-induced hyperhydration on fluid retention and endurance performance. *International Journal of Sport Nutrition and Exercise Metabolism, 17*(4), 391-410.

Goulet, E. D. B., Robergs, R. A., Labrecque, S., Royer, D., & Dionne, I. J. (2006). Effect of glycerol-induced hyperhydration on thermoregulatory and cardiovascular functions and endurance performance during prolonged cycling in a 25 degrees c environment. *Applied Physiology Nutrition and Metabolism-Physiologie Appliquee Nutrition Et Metabolisme, 31*(2), 101-109.

Goulet, E. D. B., Rousseau, S. F., Lamboley, C. R. H., Plante, G. E., & Dionne, I. J. (2008). Pre-exercise hyperhydration delays dehydration and improves endurance capacity during 2 h of cycling in a temperate climate. *Journal of Physiological Anthropology, 27*, 263-271.

Grafen, A. & Hails, R. (2002). *Modern statistics for the life sciences*. New York, USA: Oxford University Press.

Graham, G. G., Davies, M. J., Day, R. O., Mohamudally, A., & Scott, K. F. (2013). The modern pharmacology of paracetamol: Therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. *Inflammopharmacology, 21*(3), 201-232.

Hargreaves, M. (2008). Physiological limits to exercise performance in the heat. *Journal of Science and Medicine in Sport, 11*(1), 66-71.

Hasegawa, H., Takatori, T., Komura, T., & Yamasaki, M. (2006). Combined effects of pre-cooling and water ingestion on thermoregulation and physical capacity during exercise in a hot environment. [Article]. *Journal of Sports Sciences, 24*(1), 3-9.

Hessemer, V., Langusch, D., Bruck, K., Bodeker, R. H., & Breidenbach, T. (1984). Effect of slightly lowered body temperatures on endurance performance in humans. *Journal of Applied Physiology, 57*(6), 1731-1737.

Hillman, A. R. (2011). *The effects of hydration status on markers of oxidative and cellular stress during prolonged exercise in hyperthermic environments.* Degree of Doctor of Philosophy, The University of Hull, Hull, UK.

Hillman, A. R., Turner, M.C., Peart, D.J., Bray, J.W., Taylor. L., McNaughton, L & Siegler, J. (2013). A comparison of hyperhydration vs. Ad libitum fluid intake strategies on measures of oxidative stress, thermoregulation and performance. *Research In Sports Medicine, In Press*.

Hillman, A. R., Vince, R. V., Taylor, L., McNaughton, L., Mitchell, N., & Siegler, J. (2011). Exercise-induced dehydration with and without environmental heat stress results in increased oxidative stress. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme, 36*(5), 698-706.

Hillman, A. R., Vince, R. V., Taylor, L., McNaughton, L., Mitchell, N., & Siegler, J. (2011). Exercise-induced dehydration with and without environmental heat stress results in increased oxidative stress. *Applied Physiology Nutrition and Metabolism-Physiologie Appliquee Nutrition Et Metabolisme, 36*(5), 698-706.

Hitchins, S., Martin, D. T., Burke, L., Yates, K., Fallon, K., Hahn, A., & Dobson, G. P. (1999). Glycerol hyperhydration improves cycle time trial performance in hot humid conditions. [Article]. *European Journal of Applied Physiology and Occupational Physiology, 80*(5), 494-501.

Ihsan, M., Landers, G., Brearley, M., & Peeling, P. (2010). Beneficial effects of ice ingestion as a precooling strategy on 40-km cycling time-trial performance. *International Journal of Sports Physiology and Performance, 5*(2), 140-151.

Imms, F. J., & Lighten, A. D. (1989). *The cooling effects of a cold drink* (Vol. 871).

Jackson, M. J., Khassaf, M., Vasilaki, A., McArdle, F., & McArdle, A. (2004). Vitamin e and the oxidative stress of exercise. In F. Kelly, M. Meydani & L. Packer (Eds.), *Vitamin e and health* (Vol. 1031, pp. 158-168).

Jemiolo, B & Trappe, S. (2004) Single muscle fiber gene expression in human skeletal muscle: Validation of internal control with exercise. *Biochemical and Biophysical research Communications,* 320(3), 1043-1050.

Jeukendrup, A., Saris, W. H. M., Brouns, F., & Kester, A. D. M. (1996). A new validated endurance performance test. *Medicine and Science in Sports and Exercise, 28*(2), 266-270.

Kasner, S. E., Wein, T., Piriyawat, P., Villar-Cordova, C. E., Chalela, J. A., Krieger, D. W., . . . Grotta, J. C. (2002). Acetaminophen for altering body temperature in acute stroke - a randomized clinical trial. *Stroke, 33*(1), 130-134.

Kaufmann, S. H. E. (1990). Heat-shock proteins and the immune-response. *Immunology Today, 11*(4), 129-136.

Kay, D., Marino, F. E., Cannon, J., Gibson, A. S. T., Lambert, M. I., & Noakes, T. D. (2001). Evidence for neuromuscular fatigue during high-intensity cycling in warm, humid conditions. *European Journal of Applied Physiology, 84*(1-2), 115-121.

Kay, D., Taaffe, D. R., & Marino, F. E. (1999). Whole-body pre-cooling and heat storage during self-paced cycling performance in warm humid conditions. *Journal of Sports Sciences, 17*(12), 937-944.

Kellogg, D. L., Pergola, P. E., Piest, K. L., Kosiba, W. A., Crandall, C. G., Grossmann, M., & Johnson, J. M. (1995). Cutaneous active vasodilation in humans is mediated by cholinergic nerve cotransmission. *Circulation Research, 77*(6), 1222-1228.

Kenney, W. L., & Johnson, J. M. (1992). Control of skin blood-flow during exercise. *Medicine and Science in Sports and Exercise, 24*(3), 303-312.

Koennecke, H. C., & Leistner, S. (2001). Prophylactic antipyretic treatment with acetaminophen in acute ischemic stroke: A pilot study. *Neurology, 57*(12), 2301-2303.

Kuennen, M., Gillum, T., Dokladny, K., Bedrick, E., Schneider, S., & Moseley, P. (2011). Thermotolerance and heat acclimation may share a common mechanism in humans. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 301*(2), R524-R533.

Kurz, A. K., Schliess, F., & Haeussinger, D. (1998). Osmotic regulation of the heat shock response in primary rat hepatocytes. *Journal of Hepatology, 28*(SUPPL. 1), 124-124.

Latzka, W. A., & Sawka, M. N. (2000). Hyperhydration and glycerol: Thermoregulatory effects during exercise in hot climates. *Canadian Journal of Applied Physiology-Revue Canadienne De Physiologie Appliquee, 25*(6), 536-545.

Latzka, W. A., Sawka, M. N., Montain, S. J., Skrinar, G. S., Fielding, R. A., Matott, R. P., & Pandolf, K. B. (1998). Hyperhydration: Tolerance and cardiovascular effects during uncompensable exercise-heat stress. *Journal of Applied Physiology, 84*(6), 1858-1864.

Lee, J. K. W., & Shirreffs, S. M. (2007). The influence of drink temperature on thermoregulatory responses during prolonged exercise in a moderate environment. *Journal of Sports Sciences, 25*(9), 975-985.

Lee, J. K. W., Shirreffs, S. M., & Maughan, R. J. (2008). Cold drink ingestion improves exercise endurance capacity in the heat. *Medicine and Science in Sports and Exercise, 40*(9), 1637-1644.

Lee., & Haymes. (1995). Exercise duration and thermoregulatory responses after whole body precooling. *Journal of Applied Physiology, 79*(6), 1971-1976.

Lin, E. C. C. (1977). Glycerol utilization and its regulation in mammals. *Annual Review of Biochemistry, 46*, 765-795.

Linquist, S., & Craig, E., A. (1988). The heat shock proteins. *Annual Review of Genetics, 22*, 631-677.

Liu, Y., Lormes, W., Baur, C., Opitz-Gress, A., Altenburg, D., Lehmann, M., & Steinacker, J. M. (2000). Human skeletal muscle hsp70 response to physical training depends on exercise intensity. *International Journal of Sports Medicine, 21*(5), 351-355.

Locke, M., Noble, E. G., & Atkinson, B. G. (1990). Exercising mammals synthesize stress proteins. *American Journal of Physiology, 258*(4), C723-C729.

Lyons, T. P., Riedesel, M. L., Meuli, L. E., & Chick, T. W. (1990). Effects of glycerol-induced hyperhydration prior to exercise in the heat on sweating and core temperature. *Medicine and Science in Sports and Exercise, 22*(4), 477-483.

Macdougall. J. D, Reddan, W. G., Layton, C. R., & Dempsey, J. A. (1974). Effects of metabolic hyperthermia on performance during heavy prolonged exercise. [Article]. *Journal of Applied Physiology, 36*(5), 538-544.

Magalhaes, F. D., Amorim, F. T., Passos, R. L. F., Fonseca, M. A., Oliveira, K. P. M., Lima, M. R. M., . . . Rodrigues, L. O. C. (2010). Heat and exercise acclimation increases intracellular levels of hsp72 and inhibits exercise-induced increase in intracellular and plasma hsp72 in humans. *Cell Stress and Chaperones, 15*(6), 885-895.

Maloyan, A., Palmon, A., & Horowitz, M. (1999). Heat acclimation increases the basal hsp72 level and alters its production dynamics during heat stress. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 276*(5), R1506-R1515.

Marino, F. E. (2002). Methods, advantages, and limitations of body cooling for exercise performance. *British Journal of Sports Medicine, 36*(2), 89-94.

Marino, F. E., Kay, D., & Cannon, J. (2003). Glycerol hyperhydration fails to improve endurance performance and thermoregulation in humans in a warm humid environment. *Pflugers Archiv-European Journal of Physiology, 446*(4), 455-462.

Marshall, H. C., Campbell, S. A., Roberts, C. W., & Nimmo, M. A. (2007). Human physiological and heat shock protein 72 adaptations during the initial phase of humid-heat acclimation. *Journal of Thermal Biology, 32*(6), 341-348.

Martin, C., Boisson, C., Haccoun, M., Thomachot, L., & Mege, J. L. (1997). Patterns of cytokine evolution (tumor necrosis factor-alpha and interleukin-6) after septic shock, hemorrhagic shock, and severe trauma. *Critical Care Medicine, 25*(11), 1813-1819.

Mauger, A. R., Jones, A. M., & Williams, C. A. (2009). Influence of feedback and prior experience on pacing during a 4-km cycle time trial. *Medicine and Science in Sports and Exercise, 41*(2), 451-458.

Mauger, A. R., Jones, A. M., & Williams, C. A. (2010). Influence of acetaminophen on performance during time trial cycling. *Journal of Applied Physiology, 108*(1), 98-104.

Mauger, A. R., Taylor, L., Harding, C., Foster, J., & Castle, P. C. (2013). Acute acetaminophen ingestion improves time to exhaustion during exercise in the heat. *Manuscript submitted for publication*.

Mauger, A. R., Taylor, L., Harding, C., Wright, B., Foster, J., & Castle, P. C. (2013). Acute acetaminophen (paracetamol) ingestion improves time to exhaustion during exercise in the heat. *Journal of Experimental Physiology*.

Maughan, R. J., Otani, H., & Watson, P. (2012). Influence of relative humidity on prolonged exercise capacity in a warm environment. *European Journal of Applied Physiology, 112*(6), 2313-2321.

McGowan, C. (1979). Selection pressure for high body temperatures - implications for dinosaurs. *Paleobiology, 5*(3), 285-295.

Merrick, M. A., Jutte, L. S., & Smith, M. E. (2003). Cold modalities with different thermodynamic properties produce different surface and intramuscular temperatures. *Journal of Athletic Training, 38*(1), 28-33.

Milarski, K. L., & Morimoto, R. I. (1989). Mutational analysis of the human hsp70 protein - distinct domains for nucleolar localization and adenosine-triphosphate binding. *Journal of Cell Biology, 109*(5), 1947-1962.

Miller, S. L., Schopf, J. W., & Lazcano, A. (1997). Oparin's "origin of life": Sixty years later. *Journal of Molecular Evolution, 44*(4), 351-353.

Mitchell, J. B., McFarlin, B. K., & Dugas, J. P. (2003). The effect of pre-exercise cooling on high intensity running performance in the heat. *International Journal of Sports Medicine, 24*(2), 118 - 124.

Montner, P., Stark, D. M., Riedesel, M. L., Murata, G., Robergs, R., Timms, M., & Chick, T. W. (1996). Pre-exercise glycerol hydration improves cycling endurance time. *International Journal of Sports Medicine, 17*(1), 27-33.

Morimoto, R. R., Tissieres, A., & Georgopoulos, C. (1994). *The biology of heat shock proteins and molecular chaperones*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Morton, J. P., MacLaren, D. P., Cable, N. T., Bongers, T., Griffiths, R. D., Campbell, I. T., . . . Drust, B. (2006). Time course and differential responses of the major heat shock protein families in human skeletal muscle following acute nondamaging treadmill exercise. *Journal of Applied Physiology, 101*(1), 176-182.

Murray, R., Eddy, D. E., Paul, G. L., Seifert, J. G., & Halaby, G. A. (1991). Physiological-responses to glycerol ingestion during exercise. *Journal of Applied Physiology, 71*(1), 144-149.

Murray, R., Eddy, D.E., Paul, G.L., Seifert, J.G & Halaby, G.A. (1991). Physiological responses to glycerol ingestion during exercise. *Journal of Applied Physiology, 71*(1), 144-149.

Nadel, E. R., Fortney, S. M., & Wenger, C. B. (1980). Effect of hydration state on circulatory and thermal regulations. *Journal of Applied Physiology, 49*(4), 715-721.

Nakamura, K. (2011). Central circuitries for body temperature regulation and fever. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 301*(5), R1207-R1228.

Nelson, J. L., & Robergs, R. A. (2007). Exploring the potential ergogenic effects of glycerol hyperhydration. [Review]. *Sports Medicine, 37*(11), 981-1000.

Nielsen, B., Hales, J. R. S., Strange, S., Christensen, N. J., Warberg, J., & Saltin, B. (1993). Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. *Journal of Physiology-London, 460*, 467-485.

Niess, A. C., Dickhuth, H. H., Northoff, H., & Fehrenbach, E. (1999). Free radicals and oxidative stress in exercise - immunological aspects. *Exercise Immunology Review, 5*, 22-56.

Noakes, T. D. (2006). Exercise in the heat: Old ideas, new dogmas. *International Sportmed Journal, 7*(1), 58-74.

Noble, E. G., Milne, K. J., & Melling, C. W. J. (2008). Heat shock proteins and exercise: A primer. *Applied Physiology Nutrition and Metabolism-Physiologie Appliquee Nutrition Et Metabolisme, 33*(5), 1050-1065.

Northoff, H., Berg, A., & Weinstock, C. (1998). Similarities and differences of the immune response to exercise and trauma: The ifn-gamma concept. *Canadian Journal of Physiology and Pharmacology, 76*(5), 497-504.

Nybo, L. (2007). Exercise and heat stress: Cerebral challenges and consequences. *Neurobiology of Hyperthermia, 162*, 29-43.

Nybo, L., & Nielsen, B. (2001). Hyperthermia and central fatigue during prolonged exercise in humans. *Journal of Applied Physiology, 91*(3), 1055-1060.

Nybo, L. (2008) Hyperthermia and fatigue. *Journal of Applied Physiology*, 104, 871-878.

O'Brien, C., Freund, B. J., Young, A. J., & Sawka, M. N. (2005). Glycerol hyperhydration: Physiological responses during cold-air exposure. *Journal of Applied Physiology, 99*(2), 515-521.

Oishi, Y., Taniguchi, K., Matsumoto, H., Ishihara, A., Ohira, Y., & Roy, R. R. (2002). Muscle type-specific response of hsp60, hsp72, and hsc73 during recovery after elevation of muscle temperature. *Journal of Applied Physiology, 92*(3), 1097-1103.

Olschewski, H., & Bruck, K. (1988). Thermoregulatory, cardiovascular, and muscular factors related to exercise after precooling. *Journal of Applied Physiology, 64*(2), 803-811.

Peart, D. J., McNaughton, L. R., Midgley, A. W., Taylor, L., Towlson, C., Madden, L. A., & Vince, R. V. (2011). Pre-exercise alkalosis attenuates the heat shock protein 72 response to a single-bout of anaerobic exercise. *Journal of Science and Medicine in Sport, 14*(5), 435-440.

Quod, M. J., Martin, D. T., Laursen, P. B., Gardner, A. S., Halson, S. L., Marino, F. E., . . . Hahn, A. G. (2008). Practical precooling: Effect on cycling time trial performance in warm conditions. *Journal of Sports Sciences, 26*(14), 1477-1487.

Ramanathan, N. L. (1964). A new weighting system for mean surface temperature of the human body. *Journal of Applied Physiology, 19*, 531-533.

Riedesel, M. L., Allen, D. Y., Peake, G. T., & Alqattan, K. (1987). Hyperhydration with glycerol solutions. *Journal of Applied Physiology, 63*(6), 2262-2268.

Ritossa, F. (1962). A new puffing pattern induced by temperature shock and dnp in *drosphila*. *Experientia, 18*, 571-573.

Robergs, R., & Roberts, S. O. (1997). *Exercise physiology: Exercise, performance and clinical applications*. Boston (MA): WCB McGraw-Hill.

Ross, M., Abbiss, C., Laursen, P., Martin, D., & Burke, L. (2013). Precooling methods and their effects on athletic performance a systematic review and practical applications. *Sports Medicine, 43*(3), 207-225.

Ross, M. L. R., Jeacocke, N. A., Laursen, P., Martin, D. T., Abbiss, C. R., & Burke, L. M. (2012). Effects of lowering body temperature via hyperhydration, with and without glycerol ingestion and practical precooling on cycling time trial performance in hot and humid conditions. *Journal of the International Society of Sports Nutrition, 9*(55), 1-11.

Rowell, L. B. (1974). Human cardiovascular adjustments to exercise and thermal-stress. *Physiological Reviews, 54*(1), 75-159.

Rowell, L. B., Marx, H. J., Bruce, R. A., Conn, R. D., & Kusumi, F. (1966). Reductions in cardiac output central blood volume and stroke volume with thermal stress in normal men during exercise. *Journal of Clinical Investigation, 45*(11), 1801-&.

Ryan, A. J., Gisolfi, C. V., & Moseley, P. L. (1991). Synthesis of 70k stress protein by human-leukocytes - effect of exercise in the heat. *Journal of Applied Physiology, 70*(1), 466-471.

Sandstroem, M. E., Madden, L. A., Taylor, L., Siegler, J. C., Lovell, R. J., Midgley, A., & McNaughton, L. (2009). Variation in basal heat shock protein 70 is correlated to core temperature in human subjects. *Amino Acids, 37*(2), 279-284.

Santoro, G. M. (2000). Heat shock factors and the control of the stress response. *Biochemical Pharmacology, 59*, 55-63.

Sawka, M. N., Burke, L., Eichner, E. R., Maughan, R. J., Montain, S. J., & Stachenfeld, N. S. (2007). American college of sports medicine position stand. Exercise and fluid replacement. *Medicine and Science in Sport and Exercise, 39*, 377-339.

Sawka, M. N., Young, A. J., Francesconi, R. P., Muza, S. R., & Pandolf, K. B. (1985). Thermoregulatory and blood responses during exercise at graded hypohydration levels. *Journal of Applied Physiology, 59*(5), 1394-1401.

Sawka, M. N., Young, A. J., Latzka, W. A., Neufer, P. D., Quigley, M. D., & Pandolf, K. B. (1992). Human tolerance to heat strain during exercise - influence of hydration. *Journal of Applied Physiology, 73*(1), 368-375.

Schmidt, V., & Bruck, K. (1981). Effect of a precooling maneuver on body-temperature and exercise performance. *Journal of Applied Physiology, 50*(4), 772-778.

Schmittgen, T. D. L., K.J. (2008). Analyzing real-time pcr data by the comparative ct method. *Nature Protocols, 3*(6), 1101 - 1108.

Schniepp, J., Campbell, T. S., Powell, K. L., & Pincivero, D. M. (2002). The effects of cold-water immersion on power output and heart rate in elite cyclists. *Journal of Strength and Conditioning Research, 16*(4), 561-566.

Selkirk, G. A., & McLellan, T. M. (2001). Influence of aerobic fitness and body fatness on tolerance to uncompensable heat stress. *Journal of Applied Physiology, 91*(5), 2055-2063.

Shastry, S., Toft, D. O., & Joyner, M. J. (2002). Hsp70 and hsp90 expression in leucocytes after exercise in moderately trained humans. *Acta Physiologica Scandinavica, 175*(2), 139-146.

Shin, Y. O., Oh, J. K., Sohn, H. S., Bae, J. S., Lee, M. Y., Lee, J. B., . . . Matsumoto, T. (2004). Expression of exercise-induced hsp70 in long-distance runner's leukocytes. *Journal of Thermal Biology, 29*(7-8), 769-774.

Siegel, R., Mate, J., Brearley, M. B., Watson, G., Nosaka, K., & Laursen, P. B. (2010). Ice slurry ingestion increases core temperature capacity and running time in the heat. *Medicine and Science in Sports and Exercise, 42*(4), 717-725.

Siegel, R., Mate, J., Watson, G., Nosaka, K., & Laursen, P. B. (2012). Pre-cooling with ice slurry ingestion leads to similar run times to exhaustion in the heat as cold water immersion. *Journal of Sports Sciences, 30*(2), 155-165.

Skidmore, R., Gutierrez, J. A., Guerriero, V., & Kregel, K. C. (1995). Hsp70 induction during exercise and heat-stress in rats - role of internal temperature. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 268*(1), R92-R97.

Smith, J. E. (2005). Cooling methods used in the treatment of exertional heat illness. *British Journal of Sports Medicine, 39*(8), 503-507.

Szlyk, P. C., Hubbard, R. W., Matthew, W. T., Armstrong, L. E., & Kerstein, M. D. (1987). Mechanisms of voluntary dehydration among troops in the field. *Military Medicine, 152*(8), 405-407.

Taylor, L., Hillman, A., Midgley, A., Peart, D., Chrismas, B. & Mcnaughton, L. . (2012). Hypoxia mediated prior induction of monocyte expressed hsp72 and hsp32 provides protection to the sub-maximal exercise induced disturbances to redox balance. *Amino Acids, 43*, 1933-1944.

Taylor, L., Midgley, A. W., Chrismas, B., Hilman, A. R., Madden, L. A., Vince, R. V., & McNaughton, L. R. (2011). Daily hypoxia increases basal monocyte hsp72 expression in healthy human subjects. *Amino Acids, 40*(2), 393-401.

Taylor, L., Midgley, A. W., Chrismas, B., Madden, L. A., Vince, R. V., & McNaughton, L. R. (2010). Daily quadratic trend in basal monocyte expressed hsp72 in healthy human subjects. *Amino Acids, 38*(5), 1483-1488.

Taylor, L., Midgley, A. W., Chrismas, B., Madden, L. A., Vince, R. V., & McNaughton, L. R. (2010). The effect of acute hypoxia on heat shock protein 72 expression and oxidative stress in vivo. *Eur J Appl Physiol, 109*(5), 849-855.

Taylor, L., Midgley, A. W., Sandstrom, M. E., Chrismas, B., & McNaughton, L. R. (2012). The effect of the hyperbaric environment on heat shock protein 72 expression in vivo. *Research in Sports Medicine, 20*(2), 142-153.

Thomas, M. M., Cheung, S. S., Elder, G. C., & Sleivert, G. G. (2006). Voluntary muscle activation is impaired by core temperature rather than local muscle temperature. *Journal of Applied Physiology, 100*(4), 1361-1369.

Uckert, S., & Joch, W. (2007). Effects of warm-up and precooling on endurance performance in the heat. *British Journal of Sports Medicine, 41*, 380 - 384.

Vaile, J., Halson, S., Gill, N., & Dawson, B. (2008). Effect of cold water immersion on repeat cycling performance and thermoregulation in the heat. *Journal of Sports Sciences, 26*(5), 431-440.

Van Rosendal, S. P., Osborne, M. A., Fassett, R. G., & Coombes, J. S. (2010). Guidelines for glycerol use in hyperhydration and rehydration associated with exercise. *Sports Medicine, 40*(2), 113-139.

WADA. (2012). Wada: 2012 list of prohibited substances and methods

Wegmann, M., Faude, O., Poppendieck, W., Hecksteden, A., Frohlich, M., & Meyer, T. (2012). Pre-cooling and sports performance a meta-analytical review. [Review]. *Sports Medicine, 42*(7), 545-564.

Wilkinson, D. M., Carter, J. M., Richmond, V. L., Blacker, S. D., & Rayson, M. P. (2008). The effect of cool water ingestion on gastrointestinal pill temperature. *Medicine and Science in Sports and Exercise, 40*(3), 523-528.

Wingo, J. E., Casa, D. J., Berger, E. M., Dellis, W. O., Knight, J. C., & McClung, J. M. (2004). Influence of a pre-exercise glycerol hydration beverage on performance and physiologic function during mountain-bike races in the heat. *Journal of Athletic Training, 39*(2), 169-175.

Yamada, P., Amorim, F., Moseley, P., & Schneider, S. (2008). Heat shock protein 72 response to exercise in humans. [Review]. *Sports Medicine, 38*(9), 715-733.

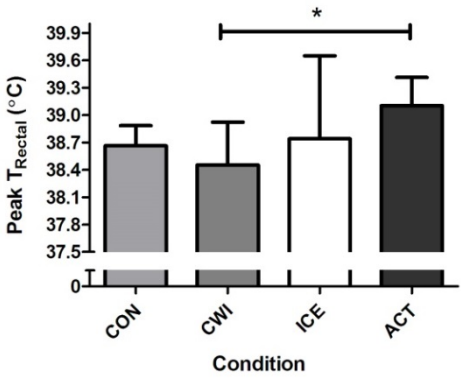
Yamada, P. M., Amorim, F. T., Moseley, P., Robergs, R., & Schneider, S. M. (2007). Effect of heat acclimation on heat shock protein 72 and interleukin-10 in humans. *Journal of Applied Physiology, 103*(4), 1196-1204.

Yoshida, T., Chida, M., Ichioka, M., & Suda, Y. (1987). Blood lactate parameters related to aerobic capacity and endurance performance. *European Journal of Applied Physiology and Occupational Physiology, 56*(1), 7-11.

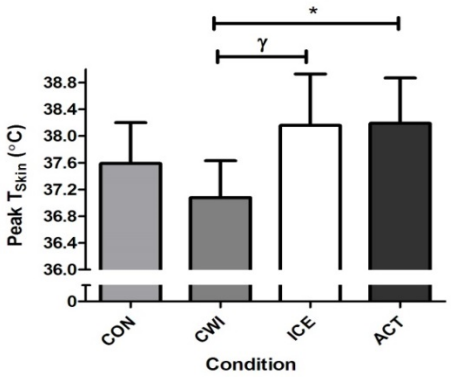
Young, A. J., Sawka, M. N., Epstein, Y., Decristofano, B., & Pandolf, K. B. (1987). Cooling different body surfaces during upper and lower-body exercise. *Journal of Applied Physiology, 63*(3), 1218-1223.

CHAPTER 9: APPENDICES

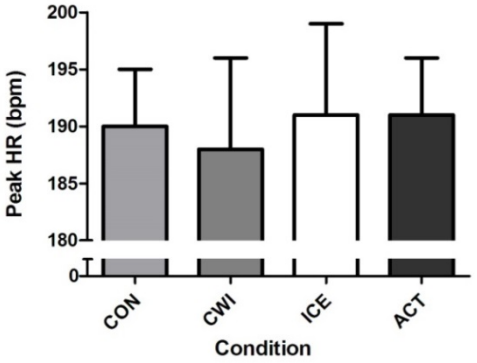
Appendix a: Peak charts for Experimental chapter 3.



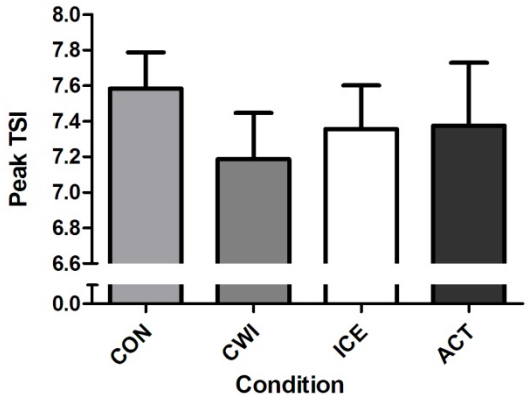
1: presented are means (*n* = 8) ± SD data for peak T_{Rectal} after exercise. * Significantly lower peak T_{Rectal} after CWI vs. ACT ($p < 0.05$).



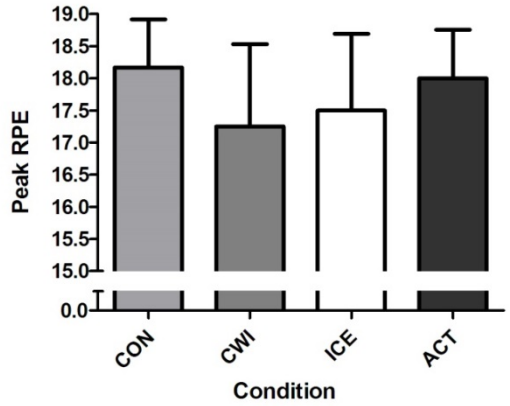
2: Presented are means (n = 8) ± SD data of peak T_{Skin} during exercise trial. * Significantly lower Peak T_{Skin} after CWI vs. ACT ($p < 0.05$) ɣ Significantly lower peak T_{Skin} after CWI vs. ICE ($p < 0.05$).



3: Presented are means (n = 8) ± SD data of peak HR during exercise trial.



4: Presented are means (n=8) ± SD data of peak RPE during exercise.



5: Presented are means (n = 8) ± SD data of peak RPE during exercise.

Appendix b - PAR – Q

1. Have you ever been told by your doctor that you have a heart condition and advised only to participate in physical activity approved by your doctor?
2. Do you experience any chest pains when you participate in physical activity?
3. Have you recently experienced any chest pains whilst not participating in physical activity?
4. Do you ever lose consciousness?
5. Do you ever lose your balance as a result of dizziness?
6. Do you have any problems with your bones and joints that could cause further problems if you participate in physical activity?

7. Are you aware of any reasons as to why you should not participate in physical activity?

Name: ..... Signature: ..... Date: .....

Appendix c - Participant Screening Form

Please read the following:

a. Are you suffering from any known active, serious infection?

b. Have you had jaundice within the previous year?

c. Have you ever had any form of hepatitis?

d. Have you any reason to think you may be HIV positive?

e. Have you ever been involved in intravenous drug use?

f. Are you a haemophiliac?

g. Is there any other reason you are aware of why taking blood might be hazardous to your health?

h. Is there any other reason you are aware of why taking your blood might be hazardous to the health of the technician?

Can you answer **Yes** to any of questions a-g? Please tick your response in the box below:

Yes ☐ No ☐

Small samples of your blood (from finger or earlobe) will be taken in the manner outlined to you by the qualified laboratory technician. All relevant safety procedures will be strictly adhered to during all testing procedures (as specified in the Risk Assessment document available for inspection in the laboratory).

I declare that this information is correct, and is for the sole purpose of giving the tester guidance as to my suitability for the test.

Name ……

Signed ……

Date ……

If there is any change in the circumstances outlined above, it is your responsibility to tell the person administering the test immediately.

The completed Medical Questionnaire (Par Q) and this Blood Sampling Form will be held in a locked filing cabinet in the Department of Sport and Exercise Science laboratories at the University for a period of one-three years. After that time all documentation will be destroyed by shredding.

Appendix d - Example information Sheet

The Combined Effect of Hyperhydration and Pre-cooling on Endurance Cycling Performance in Hot and Humid Conditions

Dear Participant,

Thank you for being interested in participating in this study. Please read the following information sheet before making the decision of whether to participate. If you decide against taking part there will be no disadvantage to you and I thank you for even considering my request.

Aim of the Project:

The purpose of this study is to evaluate the effect of pre cooling through water immersion and hyperhydration through ingestion of a glycerol and water solution on endurance cycling performance in hot and humid conditions. Subsequently looking at the thermoregulatory, metabolic and cellular responses during the exercise. This study is being undertaken as part of the requirements of a Masters (in Research) in Environmental Physiology.

Type of Participant needed:

This study is looking at competitive male cyclists and triathletes aged between 18 and 35 years old.

What the participant will have to do:

As a participant you will be asked to report to the lab on 5 separate occasions. This will consist of one familiarisation session and then 4 sessions completing the 60min time trial but after undertaking different performance enhancing strategies.

On the first visit each participant will carry out a familiarisation in temperate conditions. This involves a VO_{2max} test, a 15 minute break and then the completion of the 60 minute time trial on the Computrainer bike. On the following 4 visits to the laboratory, each subject on arrival will have their physical characteristics recorded (Height and body mass) they will also need to provide a urine sample and a fingertip blood sample.

The participant will then have to take either a glycerol solution or placebo drink. This needs to be drunk over 90 minutes following a strict schedule of how much to drink every 15

minutes. Once the 90 minutes to ingest the solution are over the participant will then have the heart rate monitor, skin thermistors and rectal thermometer attached or inserted.

The participant will rest for 5 minutes so a resting heart rate can be acquired, also they will have another fingertip blood sample to obtain the blood sample for pre exercise blood lactate. Participants will then be seated on an examination table and prepared for a venous blood sample to be taken by a qualified member of staff. When doing the control and hyperhydration only conditions the subject will then mount the bike in the environmental chamber (30ºC and 50% humidity) and do a steady 5 minutes warm up. The exercise will then commence; the participant will cycle (self paced) for 60 minutes covering as great a distance as possible. Every 5 minutes heart rate, skin temperatures and rectal temperature will be recorded. Also the participant will be asked to rate their feelings of exhaustion on a scale of 6 – 20 and rate their feelings of how hot they are on a scale of 0 – 8. Every 15 minutes a fingertip blood sample will also be taken. Once the 60 minutes have been completed, a slow cool down will commence.

Once the participant has exited the environmental chamber and rested they will have a second venous blood sample taken and will need to provide another urine sample. A final fingertip blood sample will be taken and body mass recorded. Rectal temperature will be monitored and when it has efficiently reduced back to normal the participant will be free to leave the laboratory. In the pre-cooling conditions once the glycerol or placebo solution has been drunk the heart rate monitors and rectal thermometer and skin thermistors are attached. The method of pre-cooling is via cold water immersion, so the participant will then be submerged in an inflatable water bath up to their waists with the water temperature averaging at 12ºC for 20 minutes After the duration of pre cooling the participant will get out of the tank, be towel dried, change their clothes if necessary and mount the bike to begin the warm up and exercise within 5 minutes. They will then begin the exercise as detailed above.

Possible risks of taking part in this study:

Rectal thermometer – ensure the thermometer is sterile before use. It must be inserted by the participant in the laboratory toilets but leave the door unlocked in case of emergency, they will be accompanied by an individual to provide help if they suffer from anaphylactic shock or feel faint. The insertion of the rectal thermometer should be slowly done to reduce the chances of shock. Anaphylactic shock from the rectal thermometer – this is highly unlikely to happen but to detect symptoms that it is occurring; the participant will give regular feedback on how they are feeling and if there are signs of them feeling sick and there heart rate drops dramatically in 20 -30 seconds stop the exercise immediately, lay them on the floor until they feel normal again. When the subject inserts the rectal thermometer they have to be accompanied

Contamination from blood sampling – to reduce chances of this occurring gloves will be worn when taking the sample and the area on the finger will be swabbed with an alcohol wipe to sterilise it. The lancets blades are kept in a sterile place and will be replaced if there is any contamination of them, once used they are disposed of. The venous blood sample will be attained by a qualified profession to avoid inaccurate procedure.

Fainting/discomfort in the heat – give continuous feedback regarding comfort and thermal strain from the participant, any signs of discomfort the exercise should be stopped immediately. Risk of Exertional heat illness – EHI can be easily treated if symptoms are recognised early and care given. Main symptoms are thirst, headache, dizziness, cramps and excessive fatigue to treat these the participant must be moved out of the chamber into a cooler environment, maintain normal hydration ideally with a hypertonic sports drink such as Gatorade to replenish lost electrolytes and carbohydrates.

Negative effects of increased core temperature – the experiment will be stopped if the rectal temperature rises by 2˚c from the steady resting value or rises above 39.2˚c.

Adverse reaction to glycerol – if the subject feels any of the following symptoms: nausea, headaches, dizziness or gastrointestinal distress, the exercise will be stopped. These reactions to the glycerol are highly unlikely.

Decision to withdraw from the project:

If at any stage of the project you decide you want to leave then you can. This decision is entirely up to you and there will be no disadvantage to yourself if you decide to end your participation in this study.

What will happen to the data and information collected?

Once all the testing is completed each participant will receive their own results. All information and data collected will be securely held at the University of Bedfordshire and will only be able to be accessed by relevant university staff. The results of this study may be published, but the data included will not be able to be related to you as all participants will remain anonymous.

What will I gain from taking part?

Should you choose to take part you will be entitled to a full health and fitness assessment, including a VO2max test to measure your maximal oxygen uptake, free extreme warm weather training and a range of physiological parameters measured throughout the testing.

What if I have any questions?

Any questions are welcome at any stage of the study; feel free to ask me or my supervisor: Dr. Lee Taylor at any time. Our contact details are written below.

Should you decide that you would like to participate in this study please email me and i shall send you a copy of the consent form to sign which needs to be returned before the study begins.

Many Thanks,

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